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*ANALYSIS OF VARIANCE APPLIED TO HUMAN GENETICS*

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Read before the Academy October 23, 1939

Analysis of Variance is a well-known convenient mathematical device by which the total of all squared deviations from the grand mean of some heterogeneous population may be divided into 2 variances, namely, variability about the mean of each of the more homogeneous elements in that population and the variability of these partial means about the grand mean of the entire population. It can be used to answer the question, how does the variability within the homogeneous or elementary group compare with the variability of the groups between one another? For example, how does the variability of the fruits of any one tree of a species compare with the variability of the average fruits of different trees of that species?

So far as I know the technique of Analysis of Variance has not yet been applied to human traits measured in a number of fraternities in a population. An opportunity came to me to make this application in the variance of some 14 head measurements made on the boys of 6 families, containing each from 5 to 3 boys. These dimensions were taken at age 16 years exactly or, in a few cases, by interpolation or extrapolation of a year.

The details of handling these sets of variables are well known and need not be considered here. The results are given in the accompanying table. The  $F$  values are the quotient of the larger mean square by the smaller mean square. The size of the quotient determines the chances that the  $F$  values are due merely to sampling and accordingly fail to indicate a significant difference between the two sorts of variances—within fraternities and between fraternities.

The table reveals that the mean squared variance within a family is, in all 13 traits, *less* than the variance between families, and that the ratio of one to the other is always significant and usually highly significant.

To go into some detail one may point out that the mean square variance in respect to *face width* between 16-year-old boys of one fraternity is one-twelfth that between different families even when all these families are of

"Old American" stock and when the boys in the different fraternities are being reared under practically identical conditions in an institution so that differences due to environment merely are minimal. Hence *face width*, or bizygomatic width, is determined very strongly by common intrafamilial genetical factors, while between families there are fewer common genetical factors.

In the *interpupillary distance*, similarly, the variance inside a fraternity is one-tenth that between fraternities. If one examines the entire table it appears that the lateral head dimensions—bizygomatic width, interpupillary distance, forehead width, maximum head width, distance between inner eye angles—have large  $F$  values while vertical dimensions such as elements of facial height have smaller  $F$  values. Bigonial diameter and nasion to gnathion are somewhat out of order with this general rule.

Thus it appears that the ratio of variability inside fraternities to that between fraternities is greater in lateral dimensions than in vertical dimensions; just why cannot now be said.

The Analysis of Variance illustrates the obvious fact of difference in variation (both intra- and interfraternal) according to the degree of heterogeneity of the gene complex. In monozygotic twins, between whom the genes are apparently the same, heredity influences the end result by 95 per cent or more. In general, between members of the same fraternity, more or less of the genes are unlike depending upon the greater or less remoteness of the stocks of the two parents. Where the parents are cousins variance in the fraternity is low. Where the parents are heterozygotes of two human races (as in negro-white crosses) the variances of the nasal dimensions and skin color are high.

*Between mean of fraternities of the same stable community*, more or less homogeneous in racial stock, variance will be relatively low. As the community becomes more heterogeneous mean variance between fraternities will increase and likewise the value of  $F$ . As an extreme case we can imagine a breeder of dogs, cats, guinea pigs and rabbits, who had a large litter of each species and compared variance in ear length within fraternities and between fraternities without separating species. In this example there are still fewer traits in common between the ear lengths of the species so that variance between the fraternities (and  $F$ ) would be very large. In general, if few genes are involved in building any organ interfamilial variance will not be strikingly greater than intrafamilial variance. If many genes are involved then the cluster found in one fraternity may well differ markedly from that found in another fraternity, so that interfraternal variance, and  $F$ , will be relatively large.

By the application of the method of Analysis of Variance we may in time gain some notion of relative number of genes involved in the development of an organ—or, in other words, its genetic complexity

## ANALYSIS OF VARIANCE OF HUMAN HEAD MEASUREMENTS

TRAIT	MEAN SQUARE VARIANCE		<i>F</i> VALUES
	BETWEEN FAMILIES	WITHIN A FAMILY	
Chances under 1 per 100 that <i>F</i> values are due to sampling			
Bizygomatic width	155.02	12.50	12.40
Interpupillary dist.	42.71	4.38	9.85
Minimum frontal width	123.77	14.54	8.51
Head width	98.63	13.41	7.36
Head girth	711.30	107.42	6.62
Dist. bet. inner eye ang.	17.47	2.78	6.29
Trichion to gnathion	254.19	46.59	5.46
Nasion to gnathion	129.45	24.80	5.22
Head length	80.94	16.95	4.78
Head height	54.44	12.62	4.32
Chances 1 to 5 per 100 that <i>F</i> values are due to sampling			
Bigonial diameter	70.44	18.23	3.87
Nasion to stomion	43.38	14.66	2.96
Chances over 5 per 100 that <i>F</i> values are due to sampling			
Trichion to nasion	47.77	18.01	2.65
Stomion to gnathion	31.55	13.14	2.40

*TWO X-RAY INDUCED MOSAICS IN DROSOPHILA  
PSEUDOÖBSCURA*

BY R. G. HELFER

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OF TECHNOLOGY

Communicated December 4, 1939

The mechanism of the origin of chromosomal aberrations is still an open question. The so-called contact hypothesis, advanced originally by Serebrovsky,<sup>1</sup> assumes that translocations and other gene rearrangements are formed due to chance union of chromosomes accompanied by the development of new associations between genes, somewhat in the manner of "illegitimate" crossing-over. According to this view, the breakage of the original chromosomes and the reattachment of the resulting fragments occur practically simultaneously. The alternative hypothesis assumes that chromosomes are broken first, and that some time may elapse before the points of fracture either reunite to restore the original situation or form new attachments.<sup>2,3</sup> Unfortunately, the problem is such that critical evidence has been difficult to obtain.<sup>4,5,6,7</sup> The mosaic translocations described below may possibly shed some light on the question.

Normal males of race *A* of *Drosophila pseudoöbscura* were treated with x-ray (5000) units, and outcrossed to normal untreated females. The

salivary glands of the  $F_1$  larvae were taken, stained in aceto-carmin, and permanent smear preparations were made with the aid of the usual technique. Each slide contained only the two glands of a single individual. In the course of study of these slides, two very remarkable aberrant sets of glands were found. Instead of having the customary single type of tissue, either completely normal or having all cells containing the same aberration, these two sets of glands were mosaics of more than one kind of tissue. Several facts show that this result cannot be due to contamination (i.e., mixing the glands of several individuals in the same slide). In the first place each slide contains two and only two glands; in these particular slides the two glands lie separately. Secondly, both glands of each set are of the same sex. Thirdly, and this is the main argument, both glands of each set contain mixtures of tissues, the same cytological condition being observed in some cells of either gland.

The more complex of the two sets of mosaic glands apparently contains four different tissues. An analysis was made of each of the glands of this mosaic. In one gland, a total of 42 cells proved to be satisfactorily analyzable; the precise status of 8 cells was in doubt and the rest were not clear enough to attempt a classification. The four types of cells are as follows. The first, and by far the most frequent type, observed in 58% (24 out of 42) cells examined, departs from normal in having a translocation between the third and probably the  $Y$ -chromosomes (Fig. 1*a*). In terms of the maps published by Dobzhansky and Tan,<sup>8</sup> the third chromosome is broken in section 80, between the first and the second dark discs distal to the "bulb." As the  $Y$ -chromosome in salivary glands is not a distinct body, being simply a part of the heterochromatic chromocenter, it is impossible to determine the position of the break in this chromosome. The second type of cells (11% of the total analyzed) contains a translocation involving the third and the fourth chromosomes (Fig. 1*b*). The third is broken at about the middle of section 66, and the fourth is broken in section 97, the major part of the third being exchanged for the distal end of the fourth chromosome. The third type of cell (19%) is a combination of the preceding two, i.e., the III- $Y$  translocation is present together with the III-IV one (Fig. 1*c*). Finally, the fourth type (11%) are normal cells, apparently free from any cytologically detectable abnormality. The second gland of this set contained 15 analyzable cells, 12 doubtful ones and the rest too poor for classification. Again the most frequent type of cell was that having the III- $Y$  translocation (11 out of 15). There was only one clear-cut example of the III-IV translocation, none of the III- $Y$ , III-IV; and three examples of normal third chromosomes.

Several mechanisms which may produce such a mosaic may be suggested. If one were to suppose that two sperm fertilized a single egg and that each one had one chromosome aberration in it, a mosaic individual

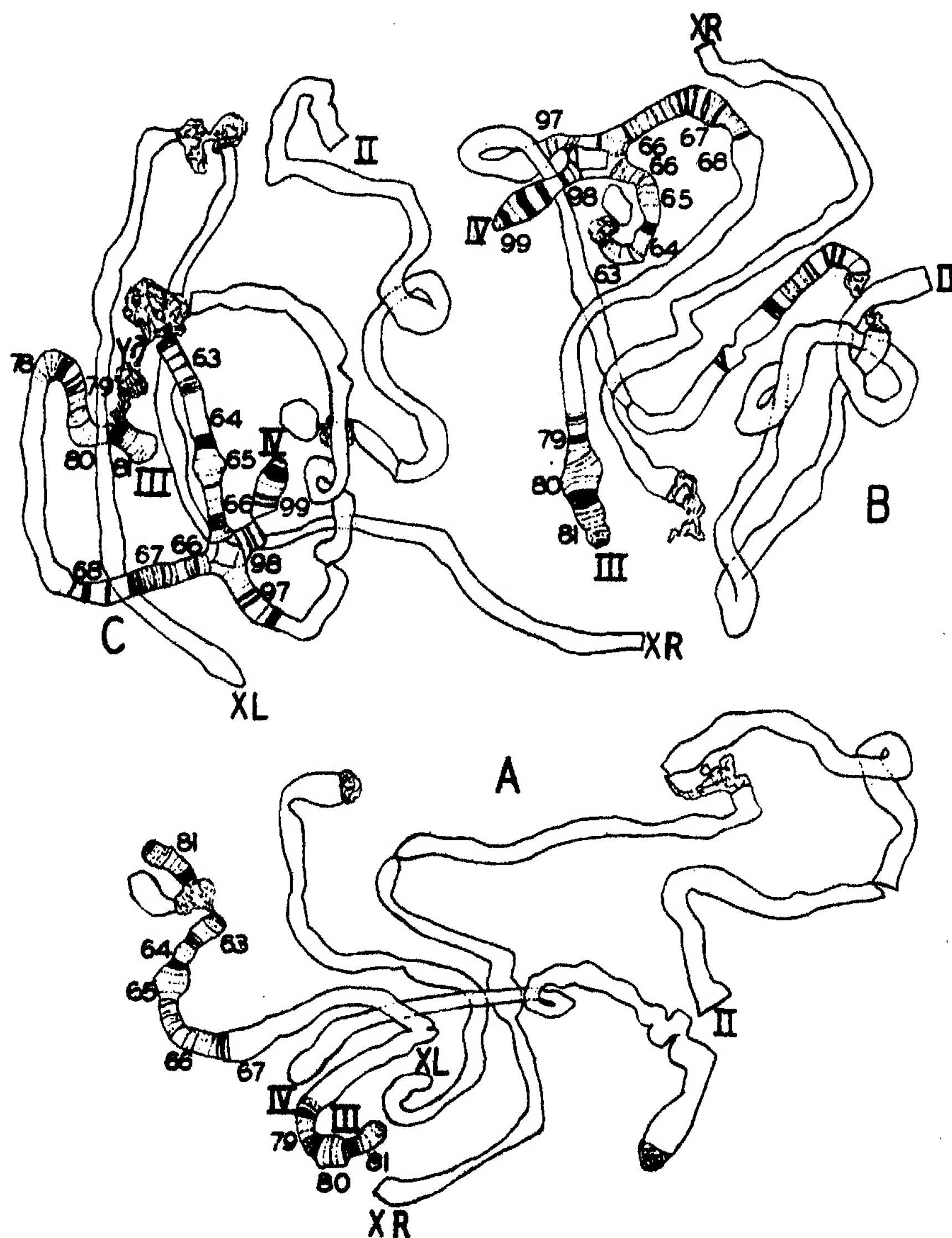


FIGURE 1

Three types of aberrant tissue found in the salivary glands of an  $F_1$  male offspring from a cross between x-rayed males to normal females. A—translocation involving the tip of III and the Y-chromosome, B—translocation between the base of III and the tip of IV, C—combination of translocations A and B.



would result. Such an individual might have two different types of salivary gland tissue. That it is possible for both nuclei of the first cleavage division to be incorporated in the salivary gland tissue is supported by recent work of Kaufmann.<sup>9</sup> The points in the present evidence which automatically rule out this hypothesis are that not two but four types of tissues are present, and that one of these contains an aberration combining the properties of the two other aberrant types (Figs. 1a and 1b). Another possibility is that x-rays as such had no effect on the sperm, but that during the course of the development three types of the aberrant tissues arose spontaneously. A spontaneous translocation has been described in an individual of *Drosophila melanogaster* that has not been treated with x-ray, and this individual has been a mosaic of normal and aberrant tissue.<sup>10</sup> Since spontaneous chromosomal changes are relatively very rare, to suppose that so rare an event takes place three times in the development of a single individual is, however, too improbable. Still another possibility is that one translocation took place due to the irradiation (for example the III-Y translocation), and then another (the III-IV translocation) occurred spontaneously after fertilization in a part of the modified tissue. This view is also ruled out because of two securely established facts, namely the presence of cells with the III-IV but without the III-Y translocation, and of the apparently normal cells containing neither translocation. A somatic crossing-over would have to be invoked to produce these additional types.

The fourth possibility is one which assumes that the chromosomes in the sperm are in the four-strand stage. Were such the case a workable hypothesis could be developed to account for the formation of a four-tissue mosaic. For example, if one supposed that the breaks induced by the x-ray at any level effect only two of the four strands present, and if in the third chromosome two of the strands are broken in region 80, whereas the other two are broken in region 66, a cross-over occurring between one strand broken at 80 and one broken at 66 would result in one unbroken normal strand, one broken both at 80 and at 66, one broken at 80 and the fourth broken at 66. Reattachment of broken parts might occur before the chromosomes went into the first cleavage spindle. Then, all these suppositions granted, the segregation in the first and the second cleavages must be such that each of the resulting four nuclei contains one of the four types of cells found. The main weakness of this hypothesis is the assumption of crossing-over among the four strands of a chromosome of a haploid group.

The fifth possibility, and the one which seems most probable to the author on the basis of the available evidence, is that the breakage of the chromosomes due to x-rays need not occur at the time of the treatment but may be delayed for one or more cell generations. Let it be assumed that the action of the x-rays has weakened, or actually broken, the third chromosome in two places, in sections 80 and in 66, the fourth chromosome in sec-

tion 97 and the Y-chromosome at an undetermined point. Such a sperm has fertilized a normal egg. During the process of the chromosome splitting in the first two cleavage divisions the weaknesses or the breaks in the chromosomes have persisted. In one of the resulting cells the broken ends have become reunited to restore the original gene arrangements, thus giving rise to cells with normal chromosomes. In one of these normal cells, before the weaknesses have become healed, an exchange has occurred between the third chromosome and the Y-chromosome. This would give rise to the III-Y aberration. In another cell, or cells, an exchange has taken place between the fragments of the third and the fourth chromosomes which is later followed by an exchange between the third and the Y-chromosomes. Thus the four types of tissue have arisen containing a III-IV translocation, a III-Y translocation and a combination of the two.

The rather involved character of the above explanation must be admitted, but it seems to be the one that best fits the observed facts. It must be noted, however, that it is not entirely unprecedented. Indeed, Lewitsky and Araratian<sup>11</sup> have described a mosaic translocation in a root of a *Crepis* seed treated with x-ray, in which some cells were normal, others contained a translocation involving certain chromosomes and still others had the chromosomes further modified, with the first modification being preserved. Lewitsky and Araratian's observations, as well as the facts presented in this article, constitute evidence in favor of the view that breakage, or "weakening" of the chromosomes due to irradiation with x-rays precedes the reattachment and formation of aberrations.

The second mosaic pair of salivary glands contained only two types of tissue. The aberrant tissue consisted of an inversion in the second chromosome from the proximal part of region 43 to the distal part of region 45. The other type of tissue was normal. In one of the two glands 32 out of 48 analyzable cells contained the aberration, in the other gland, out of 36 analyzable cells, 15 were aberrant. This mosaic is not critical as an evidence for the "breakage first" hypothesis, since any one of several mechanisms might have produced it.

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<sup>10</sup> Morgan, L. V., *Genetics*, 24, 747-752 (1939).

<sup>11</sup> Lewitsky, G. A., and Araratian, A. G., *Bull. Appl. Bot., Genetics Plant Breeding*, 27, 256-303 (1931).



## THE SIZE OF THE TOBACCO MOSAIC PARTICLE FROM X-RAY DETERMINATIONS\*

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The size of the tobacco mosaic particle has been determined by two techniques, filtration through pores of calculated dimensions and rate of fall in a field of known force. These size determinations lead to quite different results, the filtration size being larger than that estimated from the ultra centrifuge. Both methods are subject to technical difficulties and mathematical assumptions which, if not fully met, may lead to erroneous conclusions. But there are, as critical thinkers point out, even greater intrinsic difficulties with these techniques. If the ultimate virus particle is a rather small molecule which is adsorbed to a larger inert complex, both techniques will err on the side of assigning too large a size to the disease-producing particle. A quite different technique is needed to substantiate the estimated size measurement. This technique may be available in x-ray studies of the virus.

X-rays, like light, mark out the size of an object as the blacked-out area in which they are absorbed. In visible light this area is delimited on an optical micrometer. With x-rays the area may be found from the inactivation produced in the absorbing medium. The x-ray size estimate, in contradistinction to estimates based on filtration or ultra-centrifuging, is dependent on the size of the ultimate entity inactivated, not on any inert material to which it may be adsorbed. If the virus particle of tobacco mosaic is minute the rate of inactivation should be very moderate. If larger the inactivation rate should be more rapid. These rates are independent of any materials to which the virus particle might be adsorbed. The difference in rates would be present whether the true virus was free to move or fixed to inert matter.

Ordinary tobacco mosaic and several of its derivatives have been irradiated with x-rays from three metals, chromium, copper and silver. The effective wave-lengths from these tubes were 2.1, 1.5 and 0.7 Angströms.

Figure 1 shows certain of the survival curves obtained by treating tobacco mosaic and its derivatives with x-rays.

The inactivation rates of ordinary mosaic and the derivatives are essentially alike. They are concordant in showing a wave-length effect. The type of effect exhibited is explicable on the hypothesis that one absorption of x-ray energy within that portion of the particle is sufficient to inactivate it. The average size of this vital volume of the mosaic particle determined from these different wave-lengths is  $7.5 \times 10^{-10}$  cm.<sup>3</sup> The data on organic

crystals show that the interatomic distances between atoms vary between 1 and 3A with 2A as a fair average. With an atomic spacing of 2A this volume, which in our material has to do with reproduction, is equivalent to about 940,000 atoms. Seemingly highly purified materials, carrying the properties of tobacco mosaic are made up by percentage composition of 51.0 C, 7.1 H, 16.7 N, 0.5 S, 0.5 P and 2.5 carbohydrates.<sup>1</sup> The average atomic weight of this material is 16, or it corresponds rather well with that of most proteins. Multiplying the atomic volume of the portion having to do with reproduction by this value gives 15,000,000 as the molecular weight of this volume.

The estimated minimum molecular weight of the whole molecule by Svedberg<sup>2</sup> from ultra-centrifugal analysis is 17,000,000. The reproductive

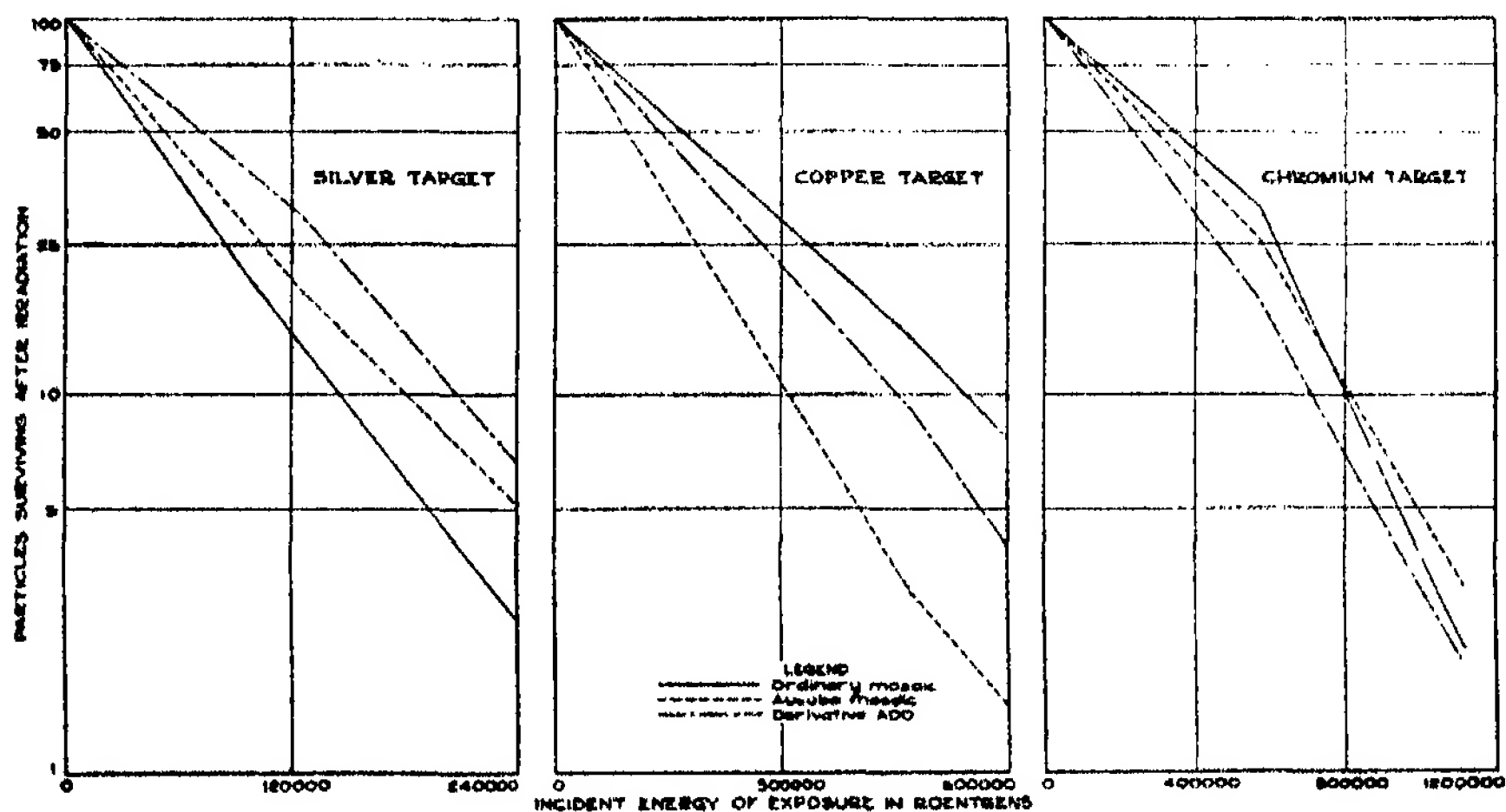


FIGURE 1

Survival curves of 3 tobacco mosaic viruses when exposed to roentgen rays of 3 wave lengths.

volume as determined above is less than this total volume but not very much less, a result which seems reasonable in view of the importance of reproduction in the organized world. The span between 15 and 17 million is left for atomic rearrangements which are not lethal to the organism. This span is known to be partly filled in as the mosaic diseases may mutate to other forms which retain the original reproductive capacity. We should expect this rate of mutation under x-rays to be quite small, an expectation which checks with our experience. Other approaches to the problem indicate that the virus entity is in the nature of a repeat molecule and may have a molecular weight of 40 to 100 million, a conclusion which gives added significance to the relative strengths of the bonds between the different molecular elements.

The data from our x-ray experiments are in agreement in viewing the tobacco mosaic virus as a rather large molecule of 16 to 20 million in molecular weight. The large portion of this molecule is important to its reproduction leaving a smaller portion capable of change without effecting this power to reproduce.

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## "PERSONALITY" DIFFERENCES AS DESCRIBED BY INVARIANT PROPERTIES OF INDIVIDUALS IN INTERACTION

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For many years, workers in social and psychological subjects have been concerned with the problem of "personality." This term is taken to mean a particular organization of behavioral elements, characteristic of the individual, which are supposed to be the product of the action of heredity and environment. Unfortunately, the discussion has been based almost entirely upon subjective impressions, and hence not only are we unable to define the properties of this entity in a precise and quantitative fashion, but also we cannot do more than hazard guesses as to the forces producing it. Attempts to define "personality" have either been in terms of a qualitative isolation of traits regarded as constituents of this entity,<sup>1</sup> or else they have been devoted, again qualitatively, to the task of describing and classifying individuals into "types."<sup>2</sup> From our own experience, we recognize that individuals differ from one another in what we call their "character" and "temperament," and we feel intuitively that this is of importance in determining how they get on with other individuals; but up to the present time, no quantitative definition has been attempted.

In a previous communication,<sup>3</sup> some preliminary results of the measurement of human interaction were presented, and it was there indicated that there are definite uniformities in the interaction of individuals. The facts described, however, were obtained by the use of a very crude measuring device which did not adequately describe the phenomena (durations of actions and inactions with two individuals). The present paper is a

report on some results obtained with an instrument with which this adequacy can be approximated. The results to be presented bear upon the problem of "personality," and a few words may help fix in the mind of the reader the significance of the procedures used.

The methods of the exact sciences are based upon the premise that relationships of functional dependence can be defined in the phenomena investigated. In order to isolate these relationships, units of measurement are defined, and the quantitative treatment consists in formulating relationships of functional dependence in terms of measured values of the variables.<sup>4</sup> If we are to use these methods, the problem of the "personality," as something apart from the influences operating on the individual, may be stated as the problem of demonstrating invariant properties of the behavior of the individual when subjected to these influences. Such individual differences have been demonstrated in a number of physiological investigations of the relationship of measured features of the performance of an organism and values of such known controlling variables as intensity of light and area on the retina,<sup>5</sup> fixed flash frequencies and intensity of light,<sup>6</sup> and so on. These differences would probably not be regarded as properties of the "personality," since "personality" is often considered to be exhibited in the relations of an individual with other individuals. If we are to demonstrate such invariant properties of the individual, they must then be isolated when that individual's performance is associated with the performance of one or more other individuals. From an operational point of view we are restricted to the observation of the interaction of individuals.<sup>7</sup>

In order to secure more accurate measurements of interaction, a recording device was constructed,<sup>8</sup> consisting essentially of a moving tape traveling at a speed of five inches *per* minute on which continuous lines are drawn by revolving, self-inking wheels. Each wheel is attached to a lever and activated independently when its key is pressed by the observer. When one individual acts, the observer presses the key assigned to that person, and the wheel lifts off the paper, not returning until the individual stops acting, when the key is released. To secure a measure of the alternation of action and inaction, one has only to measure with a scale (in seconds) the length of each inked line (inaction), and each empty space (action). By reading across from the line of one individual (*A*) to that of another (*B*), we can also obtain measures of the length of time that *A* acts and *B* is silent; when both *A* and *B* are acting (double actions); when both *A* and *B* are inactive or silent (double silence), and when individual *B* acts and *A* is silent.

The records discussed in this paper include over fifty conversations between the members of pairs of individuals, each observation lasting between thirty-five and forty-five minutes. These individuals talked in

a room, separated from the observer by a one-way screen. There were twelve individuals, not all of whom interacted with each other. Eight persons are dealt with here; the series of the other four being too short.

When frequency distributions were made of the durations of actions of an individual and again for his silences, it was seen that for both actions and silences the distributions were markedly J-shaped. To rectify the curve of the frequency,  $\log F$  was plotted as a function of  $t$  and in some instances a good fit was obtained. In the majority of cases, however, there appeared to be a definite tail to the distribution; that is, in the longer durations there was a greater observed frequency than expected. When the residuals were plotted against  $t$  it was found that this distribution was also J-shaped and a plot of  $\log F$  against  $t$  again approximated a straight line. Accordingly it was judged that the function could be fitted by the expression<sup>9</sup>

$$F = ae^{-bt} + ce^{-dt}. \quad (1)$$

For the eight individuals considered, eighty-two action and eighty-two silence distributions were plotted and the constants calculated.<sup>10</sup> In this paper we shall be concerned only with the constants  $b$  and  $d$  which define the slopes of the function. The constants  $a$  and  $c$ , which define the height of the curve in respect to the ordinate, will not be considered here.

It must be understood that a steep slope, say for actions, indicates that there are many more actions of short duration relative to the longer intervals than there are in a flat slope. For our purposes, a steep slope might have a constant whose value was  $-0.300$  or higher, while a flat slope might have a constant under  $-0.100$ . Therefore, as the long durations become exponentially more frequent relative to the short ones, the value of the constant decreases; conversely, as the short actions become more frequent, the value of the constant rises.

We have now to consider whether there is any functional dependence between the  $b$  and  $d$  slopes which we ordinarily obtain for both actions and silences, remembering that the  $b$  slope describes the exponential distribution of the shorter values, beginning with those under 1 second, and the  $d$  slope describes the frequency distribution of values running well up (in many cases) over twenty seconds—in other words, where an individual is either very silent or very talkative.

From observation, we know that individuals ordinarily exhibit a rough dependence between the durations of actions and silences; when a person becomes very talkative, for example, his silences become much shorter in duration. Since the  $b$  slopes on the one hand measure the relative distribution of long and short values within the shorter durations observed, while the  $d$  slopes measure a similar distribution in the long values, we should expect that there might be some sort of dependence between the



$b$  and  $d$  slopes. If  $b_a/b_s$  is plotted as a function of  $d_a/d_s$ , it is found that  $\log b$  ratio is a rectilinear declining function of  $\log d$  ratio, with a slope of 1.

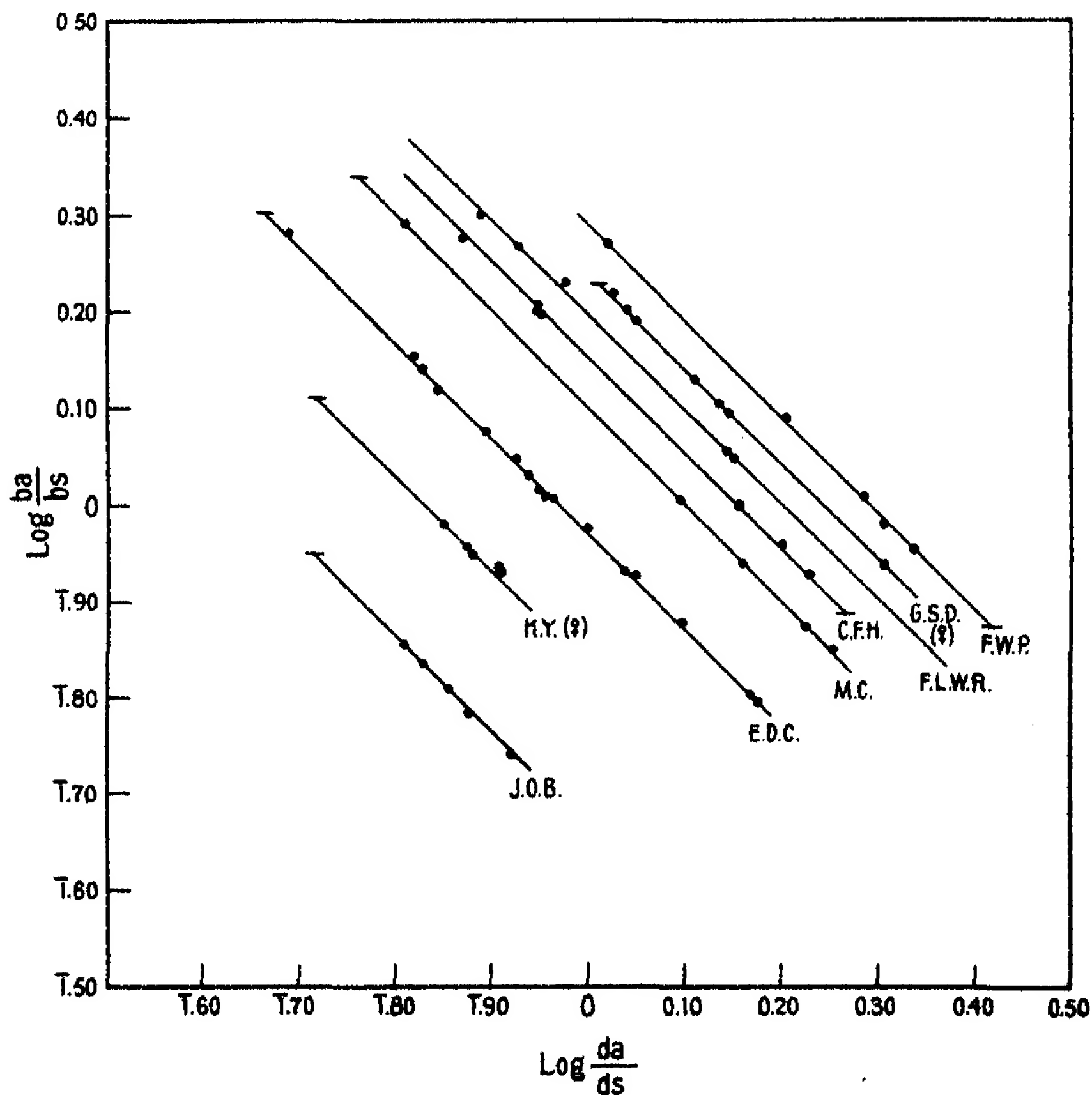
$$\log b_a/b_s = -\log d_a/d_s. \quad (2)$$

When all the cases in which an individual manifests two  $d$  slopes are plotted, it is found that all his points fall on the line defined by the above equation (2), no matter with whom he or she was interacting. In figure 1, which gives the data of eight individuals, each individual has his own curve, although the slopes ( $=1$ ) are all the same. This means that the actual rate of adjustment of the  $b$  ratio in respect to the  $d$  ratio is *unique* for each individual, although the *relative* rate is the same. In the eight curves presented, with the exception of *FLWR*, each person interacts with at least two persons, and *EDC*, the longest series, interacts with five different people. Since the position of the line does not shift in the data for different pairs including the same individual, we may consider that the position of the curve (defined by the ordinate intercept) is *invariant* for the individual.

There is additional evidence that not only the position of the curve but also its length (span) may tend to be invariant for the individual. In a small number of cases in each series, no  $d$  curve was found either in the silences or in the actions. If the position of this observation be calculated from the  $b$  ratio, it was found that in all cases in which no  $d$  slope is found (where  $N$  is large enough to expect one to occur), the value of the  $b$  ratio is either greater or less than any value in which a  $d$  ratio appears. Moreover, the absence of a  $d$  slope occurs systematically. When the value of the  $b$  ratio is less than the lowest value recorded with the  $d$  ratio, then uniformly no  $d$  silence slope appears; conversely, when the value of the  $b$  ratio is greater than any in which both  $d$  slopes appear, then no  $d$  action slope appears. Not only then is the position of the curve of this function (2) invariant on a double log grid, but also the limits within which both  $d$  slopes appear also seems to be invariant for the individual on the present evidence. In figure 1, the limits of the appearance of  $d$  ratios are fixed by lines drawn across the line of the individual at the first value of the  $b$  ratio in which no  $d$  action or silence slope appears.

The significance of this functional relationship may become clear if we regard the individual as interacting in three "states" as defined by this function. In the first, where there is no  $d$  silence curve, the value of the  $b$  ratio is below the limits defined by  $\log b$  ratio equals minus  $\log d$  ratio. This means that the  $b$  action slope is so flat and the  $b$  silence slope so steep that the individual has reached a kind of limit in talkativeness such that he does not manifest any long silences. In the other limiting case, where there is no  $d$  action slope, the value of the silences in the  $b$  slope is so low that there is no variation from a uniform rate of long silences. No long

actions sufficient to produce a  $d$  slope appear. In both these cases, however, the individual varies in his action or silent rate, that is, there is both a  $b$  and a  $d$  curve for actions if  $d$  silence is missing, and vice versa. In the intermediate state, there is dependence between four slopes, such that if the  $b$  action slope increases (more short actions appearing) and the  $b$  silence remains constant or becomes flatter, then the  $d$  action slope becomes flatter (contains relatively more long actions) or the  $d$  silence curve



becomes steeper. In other words, as a man becomes talkative he talks in longer durations, and there is a decline in the frequency of long durations and an increase in the short in the  $b$  action slope which describes the shorter actions, or else an inverse shift in the silences. Each person has his own rate of adjustment, defined by the position of the curve in relation to the intercepts.

If the  $b$  and  $d$  slopes represent processes of formation of durations at

different rates, the function (2) indicates that these processes are united in a single system, the limits of which are those cases in which at a given ratio of the  $b$  slopes, either the actions or the silences have reached a limit beyond which no  $d$  slope is formed. Thus the range between the upper and lower limits of the appearance of two  $d$  slopes defines the capacity of the individual to vary his  $d$  rates, evidenced by the appearance of  $d$  slopes. But the variation of rates so occurring is due to the process of adjustment to an individual with whom a specific individual is interacting. It can easily be seen that if neither individual interrupts the other, and if neither one is silent when the other is silent, the actions of one person will equal the silences of the other, and, conversely, and the slopes of the frequency distributions will be the same. By examination of the evidence (to be published in another place), it can be seen that this occurs very rarely. Nevertheless, there has to be a certain degree of adjustment unless one individual only talked when the other talked and was silent when the other was silent. Therefore, within the limits of adjustment of the action slopes of individual  $A$  to  $B$ 's silences and, conversely, the values of the  $d$  slopes have to be determinate. From this it follows that, in a first approximation, the limits of the appearance of a  $d$  ratio defines the capacity of an individual to vary his  $d$  rate in adjustment to another individual. An individual like  $KY$  or  $JOB$ , exhibiting a very narrow range of  $d$  ratios, is a person whose variation in response to others is such that within only a narrow range of adjustment can they manifest the long values which produce the  $d$  slope. The conditions of this adjustment will be dealt with at another time.

*Summary.*—The measurement of the interaction of individuals provides us with an opportunity to find out whether any unique property of an individual, ordinarily called "personality," manifests itself when two people are talking together. When a series of observations is made, the frequency distributions of the durations of action and silence are fitted by the exponential equation,  $F = ae^{-bt} + ce^{-dt}$ , and the plot of  $\log b_a/b_s = -\log d_a/d_s$ , with a slope of 1. The position of this curve as defined by the intercepts is invariant for each individual, since it does not shift when the individual interacts with different individuals. The range of the curve also may be invariant for each individual, being delimited at the lower end by the absence of a  $d$  silence slope and at the upper end by the absence of a  $d$  action curve. These invariant properties afford us a quantitative description of individual differences in "personality" as exhibited in the rates of acting and being silent in interaction.

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<sup>2</sup> Kretschmer, E., *Physique and Character* (trans. by W. J. H. Sprott), 2nd ed.,



London: Kegan Paul, 1936; Jung, C. G., *Psychological Types*, New York, Harcourt Brace, 1923, are well-known examples of this approach.

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<sup>4</sup> Crozier, W. J., and Hoagland, H., "The Study of Living Organisms," in Murchison, C., *A Handbook of General Experimental Psychology*, Worcester, Mass., Clark University Press, 1934, p. 3 and ff.

<sup>5</sup> Crozier, W. J., and Holway, A. H., *Jour. Gen. Physiol.*, **23**, 101 (1939).

<sup>6</sup> Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Jour. Gen. Physiol.*, **22**, 311 (1939).

<sup>7</sup> Chapple, E. D., *Measuring Human Relations* (to be published) for a discussion of this point.

<sup>8</sup> Made for us by C. A. Marston Co., Braintree, Mass.

<sup>9</sup> Lipka, J., *Graphical and Mechanical Computation*, p. 156 and ff., New York, Wiley, 1918.

<sup>10</sup> The author is greatly indebted to Gordon Donald for his assistance in calculating these slopes.

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## THE NATURE OF ABSORPTION OF RADIOACTIVE ISOTOPES BY LIVING TISSUES AS ILLUSTRATED BY EXPERIMENTS WITH BARLEY PLANTS<sup>1</sup>

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Communicated December 5, 1939

*Introduction.*—In a recent article Jenny and Overstreet<sup>2</sup> proposed a theory of ionic interchange between plant roots and soil colloids based on the assumption of partial interpenetration of the ionic double layers of root and clay surfaces. In the development of this theory of "contact exchange" the outgo of radioactive potassium from barley roots into clay suspensions and salt solutions was studied. It was found that the radioactive isotope moved readily from the roots into colloidal clay suspensions saturated with monovalent cations and to a much lesser degree into those saturated with divalent cations. This outward movement did not occur with distilled water, or with certain salt solutions investigated, of ionic content comparable to that of the colloid, with the notable exception of solutions of potassium salts. Radioactive potassium was found to move readily from barley roots into both clay suspensions saturated with potassium and solutions of potassium salts. Outward movement of radioactive potassium occurred simultaneously with the accumulation by the roots of non-radioactive potassium. This two-way movement of potassium between the roots and the bathing solutions has been interpreted as evidence for the existence of ionic exchange of cations. In this paper a more detailed examination is made of the factors governing the movement of radioactive cations between culture solutions and plant roots.

Let us consider the equilibrium distribution of any strong electrolyte  $A B$  in an inanimate system consisting of  $n$  phases. If the  $A B$  molecule dissociates into  $\nu$  ions where  $\nu = \nu_A + \nu_B$  ( $\nu_A$  = no. of cations;  $\nu_B$  = no. of anions), then the equilibrium condition for *all* phases is:

$$(a_A^{\nu_A} \times a_B^{\nu_B})_{n_1} = (a_A^{\nu_A} \times a_B^{\nu_B})_{n_2} = \dots = k. \quad (1)$$

$a_A$  and  $a_B$  are the activities of the cation and anion, respectively, and  $k$  is a constant. In the system under consideration it is assumed that the phase boundaries are permeable to all ions. However, equation (1) also has been applied to a very important class of systems in which one or more of the cations or anions are impermeable to the phase boundaries (Donnan Equilibrium). Obviously in such systems  $A B$  refers to permeable electrolytes.

If one of the cations  $A$  in the above system is a mixture of several isotopes  $A_1, A_2, A_3$ , etc., which in any phase have the isotopic mol fractions  $N_{A_1}, N_{A_2}, N_{A_3}$ , etc., at equilibrium the distribution of the isotopes will be such that:

$$\begin{aligned} (N_{A_1})_{n_1} &= (N_{A_1})_{n_2} = \dots = k_1, \\ (N_{A_2})_{n_1} &= (N_{A_2})_{n_2} = \dots = k_2, \\ (N_{A_3})_{n_1} &= (N_{A_3})_{n_2} = \dots = k_3. \end{aligned} \quad (2)$$

A distinguishing characteristic of systems in which one or more of the phases are embodied in living tissue (plant or animal cells) is the fact that the equilibrium condition represented by equation (1) rarely if ever obtains. The trend of life processes is usually away from this equilibrium condition. The accumulation of electrolytes from culture media by plant and animal cells is a well-known example of this.<sup>3</sup> On the other hand, the equilibrium distribution of isotopes represented by equations (2) is nearly always maintained in nature, even in those systems where one or more phases are present in living tissue. In general plant and animal cells are unable to bring about a separation of isotopes. From this fact it may be concluded that in the processes of metabolism and salt accumulation in plants, isotopes behave very nearly alike. The assumption seems safe that under natural conditions any plant-culture solution system is in a state of isotopic equilibrium. However, what trends may we expect if this equilibrium in such a system is deliberately disturbed, as for example by the addition of a radioactive isotope to the culture solution bathing the roots of a plant? This question is complicated by the fact that living systems are usually in a state far removed from that of thermodynamic equilibrium represented by equation (1). Jenny and Overstreet<sup>3</sup> have shown that the passage of cations between the culture medium and the root is not unidirectional (except as a net effect resulting in accumulation of cat-

ions under the influence of metabolic activities of the living cells). From this we may conclude that many if not all the phase boundaries in the plant are permeable to cations in both directions, although the permeability to an ion in one direction may be much greater than in the opposite direction. Consequently in the forementioned disturbed system, an undetermined number of phases within the plant would tend toward isotopic equilibrium with the culture medium. This would be expected even though the phases were not in thermodynamic equilibrium, since the phase boundaries are permeable to the radioactive ion in both directions. Furthermore, it is evident that the trend toward isotopic equilibrium between the culture medium and the plant root must involve a process of ionic exchange between isotopes. Thus the process is undoubtedly intimately linked to the ion exchange mechanisms of the plant. For this reason experiments were designed to observe this trend.

From the above considerations it follows that the entry of a radioactive isotope into plant roots from culture solutions will be governed by two major factors. First, the entry is dependent on the inward movement of the particular ion species to which the isotope belongs. For example, the entry of radioactive potassium is dependent on the total entry of potassium (radioactive and non-radioactive). Second, the influx is dependent on the distribution of the isotope in the various phases of the system. In other words, radioactive potassium initially in the culture solution would be expected to enter the root and move, by whatever means at its disposal, throughout the plant until the state of equal isotopic mol fractions in all phases concerned is attained. For these reasons, in order to study adequately the absorption of radioactive potassium by barley roots, it has been necessary to study the absorption of all forms of potassium.

*Experimental Technique.*—The experiments were conducted with barley plants of the Sacramento variety. The plants were grown according to the method of Hoagland and Broyer.<sup>4</sup> They are characterized by a relatively low potassium content and the roots readily accumulate potassium from very dilute solutions. Barley seeds were germinated in special chambers and transplanted into shallow pans each of which contained 3800 cc. of nutrient solution. One hundred and sixty-eight plants were set out in each pan in 24 corks. The volume of solution in the pans was maintained by the addition of distilled water daily. After approximately 3 weeks the barley plants, which had grown to about 18 inches in length, showed the first signs of starvation in the shoot, but the roots were entirely healthy ("low-salt" plants). At this stage the plants were used for experimentation. In the experiment reported here decapitated plants were used; that is, the shoots were cut off one inch above the root-stem plate. The culture solutions were analyzed for radioactivity by the method described by Jenny, Overstreet and Ayers.<sup>5</sup>

*Experimental Data.*—In the first experiment the uptake of radioactive potassium by the "low-salt" plants was studied. Groups of 84 decapitated plants were placed in shallow pans each of which contained 3000 cc. of 0.0005 *N* KCl. The KCl contained radioactive potassium, the radioactivity of the dry salt in one liter of solution being of the order of one microcurie. The plants were left in the aerated culture solutions for periods up to 9 hours. At the conclusion of each absorption period the 84 plants were removed and suitable aliquots of the culture solution were tested for radioactive potassium and analyzed for total potassium. The

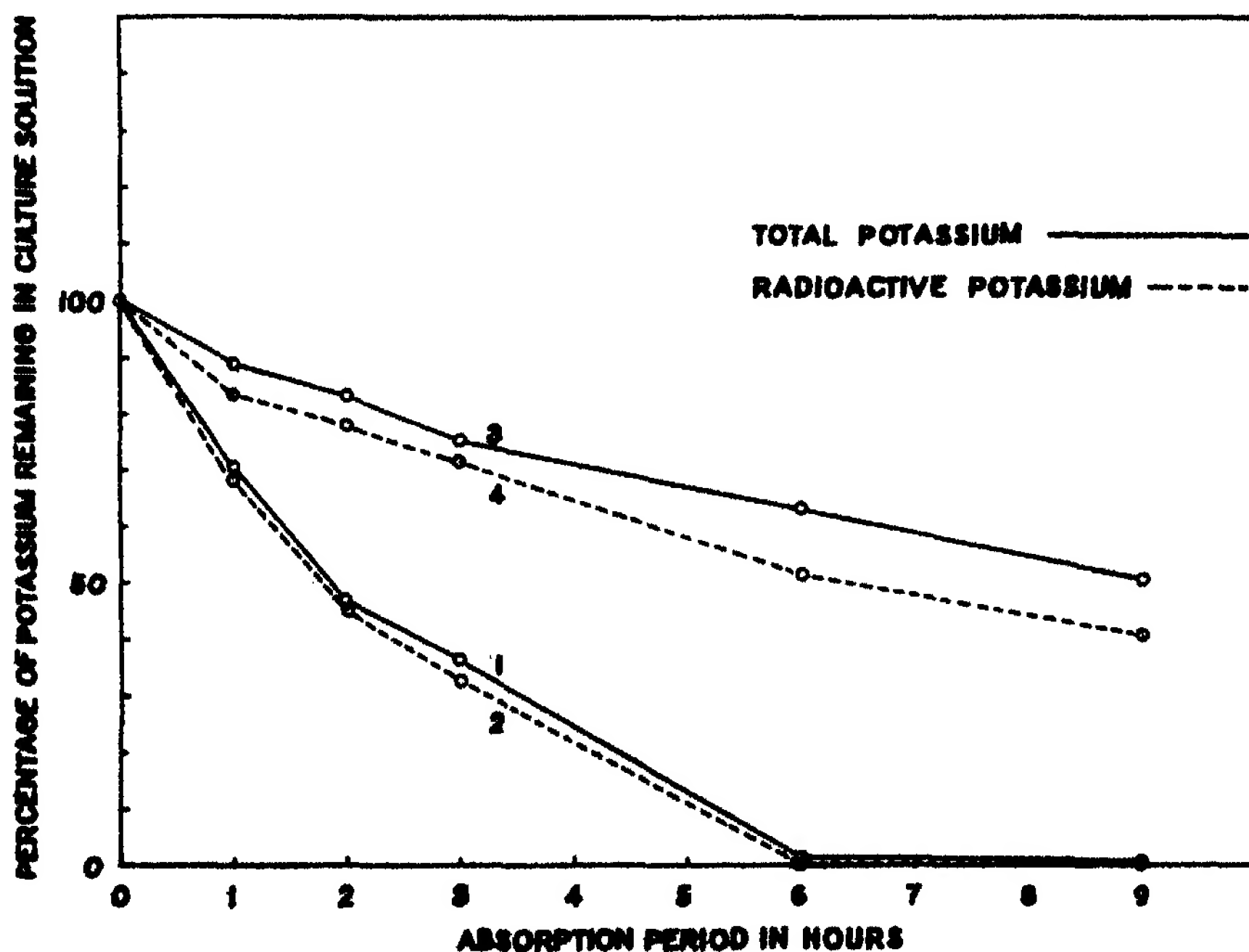


FIGURE 1

radioactive potassium and total potassium contents were each expressed as the percentages of the radioactive potassium and total potassium contents of the original culture solution. These percentages are plotted against the absorption times in figure 1, curves 1 and 2. The graph shows that the fractional uptake of the radioactive isotopes is not significantly different from that of the non-radioactive isotopes. No trend toward isotopic equilibrium can be detected with the "low-salt" plants since the capacity for accumulation of all kinds of potassium is so large. It is apparent from curves 1 and 2, figure 1, that the plant cannot distinguish between radioactive and non-radioactive isotopes in absorption, within the limits of error of this experiment.

From the data of the foregoing experiment it seemed probable that in order to observe the trend toward isotopic equilibrium it would be necessary to reduce the total accumulation of potassium. With this point in view the following experiment was run. "Low-salt" barley plants were allowed to absorb salt for a period of 19 hours from a nutrient solution containing a relatively high amount of non-radioactive potassium. During this period the potassium content of the roots rose from 34.7 to 131 milliequivalents per 100 grams oven-dry material (the average oven-dry weight of the roots from 84 plants was 3.75 grams). The shoots were then cut off and groups of 84 plants allowed to absorb KCl containing radioactive potassium under exactly the same conditions as in the first experiment. The percentages of total and radioactive potassium remaining in the culture solution are plotted in figure 1, curves 3 and 4. Examination of the curves reveals that the pretreatment has greatly reduced the rate of accumulation of total potassium as well as the accumulation of radioactive potassium. The significant divergence of curves 3 and 4 shows that the pretreated plants exhibit a preferential absorption of the radioactive isotope. This would be expected from the considerations mentioned above in regard to isotopic equilibrium.

An attempt was then made to produce roots which would show no increase in total potassium when immersed in dilute KCl solutions containing radioactive potassium. Under these conditions the only possible absorption reaction would be an ionic exchange between potassium of the culture solution and potassium of the root. To this end, barley plants were grown for 3 weeks in complete nutrient solution which was renewed 3 times a week. The decapitated plants were then immersed in radioactive KCl solutions as before. The results are given in table 1.

TABLE 1

ABSORPTION OF K FROM 0.0005 N KCl SOLUTIONS (3 LITERS) CONTAINING K<sup>42</sup> BY DECAPITATED "HIGH-SALT" BARLEY PLANTS

ABSORPTION PERIOD IN HOURS	% OF ORIGINAL K REMAINING IN CULTURE SOLUTION	% OF ORIGINAL K <sup>42</sup> REMAINING IN CULTURE SOLUTION	RADIOACTIVITY OF DRY ASH OF ROOTS IN COUNTS PER MIN.
0	100.0	100.0	0
1	112.2	95.7	136
2	110.8	97.3	167
3	110.8	91.0	289
6	109.9	93.7	278
9	119.4	91.0	629

Average oven-dry weight of roots from 84 plants = 2.65 gm.

Original total K content of roots of 84 plants = 0.145 gm.

Table 1 reveals the following facts. The "high-salt" roots actually decreased in total potassium, yielding potassium to the culture solutions. At the same time the roots absorbed radioactive potassium. This absorption

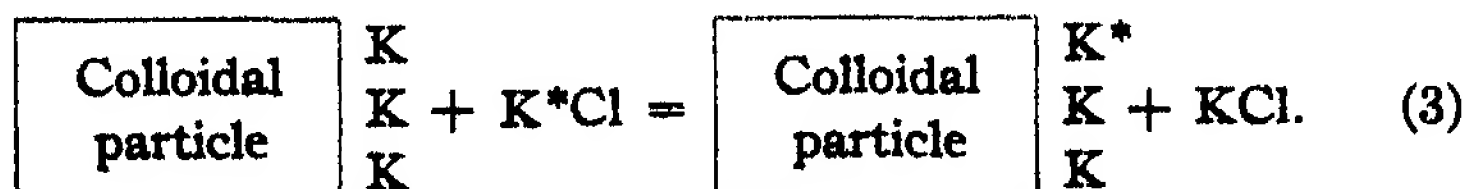


of radioactive potassium by the roots is brought out more significantly by the counts on the dry ash of the roots. The results are shown in the fourth column of table 1, in which the counts per minute on the dry ash of each set of 84 plants are given. There occurred a continuous exchange of radioactive potassium of the culture solution for non-radioactive potassium of the root throughout the 9-hour absorption period. The question arises now as to the extent to which this simple exchange process between culture solution and plant would go if given longer periods of time. It is possible that eventually all parts of the plant would come to isotopic equilibrium with the culture solution, but certain experiments with barley plants at low temperatures indicate that, at least for those conditions, such isotopic equilibrium may never be reached within the life period of the tissues. At 0°C. barley plants accumulate potassium from KCl solutions in very minute amounts if at all. On the other hand, it has been found in another research (Broyer and Overstreet) that the exchange of radioactive potassium between plant roots and culture solution is little affected by temperature. Thus for even "low-salt" barley plants placed in dilute KCl solutions containing radioactive potassium, the major process occurring at low temperatures is a simple exchange of radioactive potassium of the culture solution for ordinary isotopes of potassium initially present in the plant. In an experiment to be reported more fully elsewhere (Broyer and Overstreet) sets of 7 barley plants each were placed in 400 cc. of 0.000455 *N* KCl solution containing radioactive potassium at 0°C. After various absorption periods from 1/2 minute to 3 hours the roots were removed from the culture solution, washed, ashed and counts for radioactivity made. The results are shown in figure 2 in which the counts per minute for the roots of the seven plants are plotted against the absorption time. The absorption of radioactive potassium tended toward a maximum indicating that under these conditions only a limited part of the plant may be involved in the exchange process.

*Discussion.*—The experiments show that "low-salt" barley plants (containing approximately 30 milliequivalents potassium per 100 gm. dry weight of roots) at normal temperature absorb radioactive potassium and non-radioactive potassium from culture solutions in very nearly constant proportions. These plants apparently do not distinguish between the radioactive and non-radioactive isotopes in absorption from solutions of the concentrations indicated, but barley plants of high potassium level definitely do not absorb the isotopes in constant proportions. With these latter plants, the absorption of the radioactive isotope is favored. This fact is in harmony with the picture of a trend toward isotopic equilibrium between the culture solution and plant roots.

The trend toward isotopic equilibrium involves in essence a simple exchange between radioactive isotopes of the outside medium for non-radio-

active isotopes of the plant. If we assume the plant root to include a system of colloidal particles possessing adsorbed potassium ions, the process may be indicated by the equation:



As may be seen from equation (3), the reaction involves no change in the total amount of potassium adsorbed on the colloidal particles. This phenomenon presents a serious difficulty in the quantitative interpretation of data concerning radioactive elements, which becomes apparent from the data cited on experiments with potassium ions. For example, in one instance (Fig. 1, curves 3 and 4) both radioactive and non-radioactive potassium enter the plant in proportions which vary with time, while in another instance (Fig. 2) there is a movement of radioactive potassium into the plant with no *net* movement of potassium in the plant-solution culture system. A still more striking case is described in table 1, in which a movement of radioactive potassium into the plant is accompanied by a net release of potassium from the plant. In the light of the above information it seems imperative that conclusions in regard to net movements of an ion initially present in the organism based on observations of the movements of its radioactive isotope must be made with caution. This is especially true in the absence of information on the movements of the non-radioactive isotopes, and on the original salt status of the living material. The absorption of radioactive ions, while of great interest in relation to intermediate steps in the process of net accumulation of ions involving metabolically controlled cell activities is not a measure of this accumulation.

Experiments with barley roots indicate that under low temperature conditions only a certain part of the root is capable of attaining isotopic equilibrium with the outside medium within moderate time periods. This part may represent the colloidal phases of the protoplasm and cell wall which are capable of rapid ionic exchange with the outside solution. It is of interest to calculate the total potassium associated with this fraction from the data for figure 2. At the maximum of the curve, 1350 counts for the roots of 7 plants, it will be assumed that a certain fraction of the potassium of the roots is in isotopic equilibrium with the potassium of the culture solution. Thus for the two quantities of potassium involved, the ratio of radioactive potassium to total potassium must be the same. At the equilibrium point the outside culture solution contained 7.11 mg. potassium and the total count of radioactivity on a basis comparable to that used in estimating radioactivity in the roots was 50,900 counts per minute. From these data it follows that 0.188 mg. of the total potassium present in the seven plants is capable of isotopic equilibrium with the outside solution.

Since the oven-dry weight of the roots of seven plants was 0.17 gm. this potassium corresponds to about 2.8 milliequivalents per 100 gm. of the oven-dry roots. The total potassium content of the roots was approximately 30 milliequivalents per 100 gm. of oven-dry material. Consequently, roughly 10% of the total potassium in the roots of "low-salt" barley plants is exchangeable at 0°C. Since the effect of temperature upon the exchange reaction is known to be slight, this percentage can be assumed to represent that fraction of potassium in the roots at normal temperatures which is capable of rapid exchange for isotopes in the bathing culture medium. This value has the same order of magnitude as that for potassium

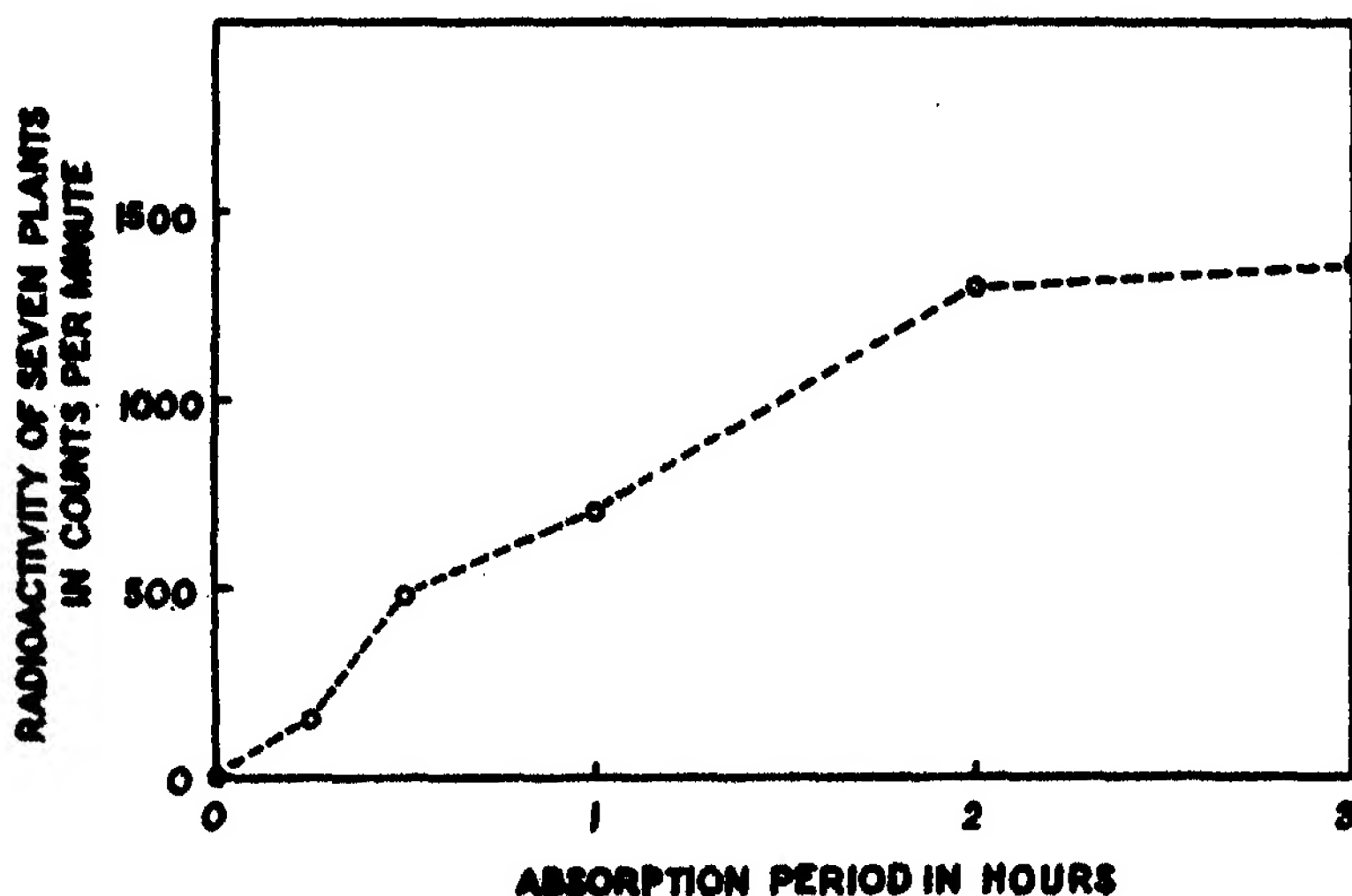


FIGURE 2

not easily recoverable by sap expression methods (unpublished data, Broyer and Hoagland).

*Summary.*—From considerations of equilibria, it is concluded that plant roots may absorb radioactive ions by means of a simple exchange of radioactive isotopes of the surrounding culture medium for non-radioactive isotopes initially present within the plant.

Barley plants with low potassium levels absorb radioactive and non-radioactive isotopes of potassium in nearly constant proportions from dilute KCl solutions in which the radioactivity of the dry salt is of the order of one microcurie per liter. Under similar conditions, barley plants with moderately high potassium levels favor the radioactive isotope in absorption.

Under the conditions studied, plants with high potassium levels or plants maintained at low temperatures do not show a net absorption of potassium. However, they do absorb radioactive potassium, indicating a process of



exchange of radioactive isotopes in the culture medium for non-radioactive isotopes in the roots.

The fraction of potassium present in the root capable of rapid exchange for isotopes in the culture medium is calculated. This fraction is believed to be associated with the colloidal phases of the protoplasm and cell wall, and for this reason may have a special significance for the study of certain aspects of the ionic interrelations of the root and culture medium.

*Acknowledgments.*—The authors are indebted to Professors D. R. Hoagland and H. Jenny for their interest and valuable suggestions, and to Professor E. O. Lawrence, Director of the Radiation Laboratory, for supplying radioactive potassium.

<sup>1</sup> Assistance in the preparation of the experimental material was furnished by the personnel of Works Progress Administration Official Project No. 465-03-3-587.

<sup>2</sup> Jenny, H., and Overstreet, R., *Proc. Nat. Acad. Sci.*, 24, 384 (1938).

<sup>3</sup> Compare Brooks, S. C., *Protoplasma*, 8, 389 (1929).

<sup>4</sup> Hoagland, D. R., and Broyer, T. C., *Plant Physiol.*, 11, 471 (1936).

<sup>5</sup> Jenny, H., Overstreet, R., and Ayers, A. D., *Soil Sci.*, 48, 9 (1939).

<sup>6</sup> The star is used to designate the radioactive isotope of potassium.

## CONCERNING THE OPEN SUBSETS OF A PLANE CONTINUUM

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In this paper, two theorems relating to unbounded plane continua will be established.

**THEOREM 1.** *If, in a plane  $S$ ,  $K$  is a closed point set and  $G$  is a countable set of mutually exclusive continua and  $G^*$  (the sum of all the continua of the set  $G$ ) has no point in common with  $K$  and  $G^* + K$  is a continuum then every element of  $G$  is a component of  $G^*$ .*

*Proof.* Let  $M$  denote  $G^* + K$ . Suppose  $K$  has a bounded component  $H$ . Then there exists a bounded domain  $D$  containing  $H$ . Let  $L$  denote the component of  $M \cdot \overline{D}$  that contains  $H$ . The boundary of  $D$  contains a point  $B$  of  $L$ . Thus the connected subset  $L$  of  $M$  contains at least one point  $B$  not belonging to  $H$ . Hence  $L$  is not a subset of  $K$ . Let  $W$  denote the collection whose elements are  $K$  and the continua of the set  $G$ . Let  $Q$  denote the set of all point sets  $q$  such that  $q$  is the common part of  $L$  and some point set of the collection  $W$ . The collection  $Q$  is a non-degenerate countable collection of mutually exclusive closed point sets filling up the compact continuum  $L$ . But this is contrary to a theorem of Sierpinski's.<sup>1</sup> It follows that every component of  $K$  is unbounded.

If there were only one continuum in the collection  $G$  then  $M$  would be the sum of two mutually exclusive closed point sets  $K$  and  $M-K$ , contrary to the supposition that it is a continuum. Hence there exist two continua  $x$  and  $y$  belonging to  $G$ . There exists an arc  $AB$  from a point  $A$  of  $x$  to a point  $B$  of  $y$ . With the aid of the above-mentioned theorem of Sierpinski's, it follows that  $AB$  contains a point  $O$  not belonging to  $M$ . Let  $T$  denote an inversion of the plane  $S$  about some circle with center at  $O$ . Let  $g$  denote a continuum of the collection  $G$  and let  $N$  denote the component of  $M-K$  that contains  $g$ . Suppose  $N$  is distinct from  $g$ . Then there exists an infinite subcollection  $G_1$  of  $G$  such that  $N$  is the sum of all the continua of  $G_1$ . The point set  $T(N)$ , the image of  $N$  under the inversion  $T$ , is bounded and connected. Since every component of the closed point set  $K$  is unbounded,  $O + T(K)$  is a compact continuum. Let  $I$  denote the complementary domain of  $O + T(K)$  that contains  $T(N)$ . The boundary of  $I$  is a subcontinuum  $\beta$  of  $O + T(K)$  and  $T(N) + \beta$  is closed. There exists a reversibly continuous transformation  $Z$  throwing the simply connected domain  $I$  into the plane  $S$ . The image of  $T(N)$  under this transformation is a plane continuum which is the sum of a countable number of mutually exclusive continua. But this involves a contradiction.<sup>2</sup> It follows that  $N$  is identical with  $g$  and, therefore, that every element of  $G$  is a component of  $M-K$ .

It has been shown by W. T. Reid<sup>3</sup> that if  $K$  is a proper subcontinuum of a plane continuum  $M$  then  $K$  contains a limit point of some component of  $M-K$ . This proposition does not remain true if the requirement that  $K$  be a proper subcontinuum of  $M$  is replaced by the weaker requirement that it be a closed subset of  $M$ . Indeed the following theorem holds true.

**THEOREM 2.** *There exists a plane continuum  $M$  containing a closed point set  $K$  such that  $M-K$  has only a countable number of components and  $K$  contains no limit point of any one of them.*

*Proof.* It has been shown by Mazurkiewicz<sup>4</sup> that there exists a plane continuum  $M$  which is the sum of a countable number of mutually exclusive closed point sets all but one of them being continua. Let  $K$  denote the one which is not a continuum and let  $G$  denote the collection of all the others. By Theorem 1, every continuum of the collection  $G$  is a component of  $M-K$ . But  $K$  contains no limit point of any continuum of that collection.

<sup>1</sup> W. Sierpinski, "Un theoreme sur les continus," *Tohoku Math. Jour.*, 13, 300-303 (1918).

<sup>2</sup> That no plane continuum is the sum of a countable number of mutually exclusive continua has been shown both by Mazurkiewicz and by the author. Cf. *Fundamenta Mathematicae*, 5, 188-205 and 6, 189-202.

<sup>3</sup> "A Theorem on Plane Continua," *Bull. Amer. Math. Soc.*, 41, 684-688 (1935).

<sup>4</sup> Loc. cit.

## ON HOMOTOPY AND EXTENSION OF MAPPINGS

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The purpose of this note is to exhibit a relation between certain special homotopy properties and the problem of extending mappings.<sup>1</sup> We consider only separable regular spaces, i.e., homeomorphs of subsets of the Hilbert parallelotope. Mapping means continuous mapping.

**THEOREM.** *If  $A$  is a compact set and  $Y$  is an absolute neighborhood retract then a necessary and sufficient condition for a mapping  $f \in Y^A$  to be homotopic to a constant is that  $f$  be extendable (relative to  $Y$ ) to every space  $X$  which contains  $A$ .*

Since a constant mapping can be extended to every space  $X$  the necessity is a consequence of the theorem. If mappings  $f$  and  $g \in Y^A$  are homotopic then, for any  $X$  containing  $A$ ,  $f$  can be extended to  $X$  if and only if the same is true of  $g$ . Interpreting homotopy of  $f$  and  $g$  as the existence of a continuous curve in  $Y^A$  containing  $f$  and  $g$ , this is easily seen to be a consequence of the Borsuk theorem.<sup>2</sup> The set  $\Pi(Y^A, X)$  of mappings of  $Y^A$  which can be extended to  $X$  is open and closed in  $Y^A$ .

The proof of the sufficiency of this condition does not require anything of the separable, regular spaces  $A$  and  $Y$ . Let  $X$  be the Hilbert parallelotope so that by hypothesis there is a mapping  $f^* \in Y^X$  such that  $f^*(x) = f(x)$  for every  $x \in A$ . Since  $A$  is contractible in  $X$  there is a mapping  $h \in X^A \times [0, 1]$  and a point  $p \in X$  such that  $h(x, 0) = p$  and  $h(x, 1) = x$ . Then  $f^*h \in Y^A \times [0, 1]$  and  $f^*h(x, 0) = f^*(p) \in B$  and  $f^*h(x, 1) = f^*(x) = f(x)$ . Thus  $f^*h$  is a homotopy in  $Y$  between  $f$  and a constant.

By specializing  $A$  to a subset  $B$  of  $Y$  and  $f$  to the identity we have

**COROLLARY.** *If  $B$  is a closed subset of an absolute neighborhood retract  $Y$  then  $B$  is contractible in  $Y$  if and only if for every space  $X$  containing  $B$  there is a mapping  $f^* \in Y^X$  under which every point of  $B$  is fixed.*

An equivalent formulation is easily seen to be the following:

*If  $B$  is a closed subset of an absolute neighborhood retract  $Y$  then  $B$  is contractible in  $Y$  if and only if for every space  $X$ , closed subset  $A$  and mapping  $f \in B^A$  there is an extension of  $f$  to  $X$  relative to  $Y$ .*

Further specializing  $A$  to be equal to  $Y$  yields the classical theorem.<sup>2</sup>

*In order that an absolute neighborhood retract be contractible it is necessary and sufficient that it be an absolute retract.*

Obviously the corollary permits one to replace any statement about sets contractible in an absolute neighborhood retract  $Y$  by a statement about fixed points under mappings into  $Y$ . Thus in particular

The category,<sup>1</sup>  $\text{cat } Y$ , of an absolute neighborhood retract  $Y$  imbedded in the Hilbert parallelotope  $X$  is the least integer  $k$  for which there are  $k$  mappings  $f_1, f_2, \dots, f_k \in Y^X$  such that every point of  $Y$  is fixed under one of these mappings.

This result, which might serve as the definition of category, is of particular interest as it exhibits the category as a generalization of the notion of absolute retract.

An equivalent formulation is the following generalization of a Borsuk theorem.<sup>2</sup>

The category,  $\text{cat } Y$ , of an absolute neighborhood retract  $Y$  is the least integer  $k$  such that, given any space  $X$  and closed subset  $A$  and mapping  $f \in Y^A$ , there are  $k$  mappings  $f_1, f_2, \dots, f_k \in Y^X$  such that for every  $x \in A$  there is an index  $i$  for which  $f_i(x) = f(x)$ .

Elsewhere,<sup>4</sup> I have introduced the concept of  $n$ -homotopy. Mappings  $f$  and  $g \in Y^A$  are said to be  $n$ -homotopic if the continuous complexes  $f\phi$  and  $g\phi \in Y^P$  are homotopic for every at most  $n$ -dimensional continuous complex  $\phi \in A^P$ . I have raised the question<sup>5</sup> of the extendability of a mapping which is  $n$ -homotopic to an extendable mapping. Our only theorem in this connection concerns the converse problem.

If  $A$  is a compact set and  $f$  is a mapping  $\in Y^A$ , a sufficient condition for  $f$  to be  $n$ -homotopic to a constant is that  $f$  be extendable to every at most  $(n + 1)$ -dimensional compact space  $X$  which contains  $A$ .

According to Borsuk<sup>6</sup> there is an  $(n + 1)$ -dimensional infinite complex  $P_{n+1}$  such that  $X = A + P_{n+1}$  is compact and  $LC^n$  and every at most  $n$ -dimensional continuous complex in  $X$  is homotopic to a constant. Hence the identity mapping,  $1$  of  $A$  is  $n$ -homotopic in  $X$  to a constant. If  $f^*$  is an extension of  $f$  to  $X$  then  $f^*1 = f$  is  $n$ -homotopic in  $Y$  to a constant.

Returning to the situation in the second formulation of the corollary we consider spaces  $X$  and  $Y$  and a mapping  $f$  of a closed subset  $A$  of  $X$  into a subset  $B$  of  $Y$ . We have found conditions under which the existence of an extension  $f^*$  of  $f$  to  $X$  relative to  $Y$  is assured. We shall now look for conditions which guarantee the existence of an  $f^*$  which has the additional property that it maps  $X - A$  into  $Y - B$ . Such an extension has been called, by Lefschetz,<sup>7</sup> an *expansion*. The use which Lefschetz makes of expansion rests on the following remark:

If  $f$  and  $g$  are mappings  $\in B^A$ ,  $f^*$  is an expansion of  $f$  to  $X$  relative to  $Y$  and  $g^*$  is an extension of  $g$  to  $X$  relative to  $B$  then  $f^*$  and  $g^*$  have the same coincidences as  $f$  and  $g$ . Explicitly:  $f(x) = g(x)$  if and only if  $f^*(x) = g^*(x)$ .

When  $Y$  is the unit segment  $[0, 1]$  and  $B$  is the union  $[0] + [1]$  of its end-points, any  $f$  has an expansion<sup>8</sup> given by

$$f^*(x) = \frac{\rho(f^{-1}(0), x)}{\rho(f^{-1}(0), x) + \rho(x, f^{-1}(1))},$$

where  $\rho$  is a metric in  $X$ .

To prove a general theorem on expansion we introduce the following terminology: A subset  $B$  of a space  $Y$  is *deformable* in  $Y - B$  if there is a mapping of the cylinder  $B \times [0, 1]$  into  $Y$  which leaves every point of  $B = B \times [0]$  fixed and maps the remainder  $B \times (0, 1]$  into  $Y - B$ . If, in addition,  $B \times [1]$  is mapped into a single point,  $B$  will be said to be *contractible* in  $Y - B$ .

*A necessary and sufficient condition for a subset  $B$  of a space  $Y - B$  to be deformable in  $Y - B$  is that for every space  $X$  and retract  $A$  of  $X$  any mapping  $f \in B^A$  can be expanded to  $X$  (relative to  $Y$ ).*

The sufficiency of the condition follows from the definition of deformation in  $Y - B$  on choosing  $X = B \times [0, 1]$  and  $A = B \times [0]$ .

To prove the necessity we define the following functions:

- (a)  $r \in A^X$  such that  $r(x) = x$  for every  $x \in A$ .
- (b)  $h \in Y^{B \times [0, 1]}$  such that  $h(y, 0) = y$  and  $h(y, t) \in Y - B$  for every  $(y, t) \in B \times (0, 1]$ .
- (c)  $u \in [0, 1]^X$  such that  $u^{-1}(0) = A$ ;  $u$  may be defined explicitly by  $u(x) = \lambda \rho(x, A)$  with  $\lambda$  a suitable positive number. We then define  $f^* \in Y^X$  by

$$f^*(x) = h(f(r(x)), u(x))$$

and observe that  $f^*$  is the desired expansion.

Slight modification of the previous proof is all that is required to obtain either of the following two theorems:

*A necessary and sufficient condition for a subset  $B$  of a space  $Y$  to be contractible in  $Y - B$  is that for every space  $X$  and neighborhood retract  $A$  of  $X$  any mapping  $f \in B^A$  can be expanded to  $X$  (relative to  $Y$ ).*

*A necessary and sufficient condition for a subset  $B$  of a space  $Y$  to be deformable in  $Y$  into another subset  $B'$  is that, for every space  $X$ , retract  $A$  of  $X$  and neighborhood  $U$  of  $A$  in  $X$ , any mapping  $f \in B^A$  has an extension  $f^* \in Y^X$  such that  $f^*(X - U) \subseteq B'$ .*

<sup>1</sup> For the terms used the reader is referred to the papers of Borsuk, especially *Fund. Math.*, 17, 152-170 (1931); 19, 220-242 (1932); 26, 123-136 (1936).

<sup>2</sup> *Fund. Math.*, 19, 229.

<sup>3</sup> *Fund. Math.*, 17, 161.

<sup>4</sup> "On the Lusternik Schnirelmann Category," to appear in *Ann. Math.*, §13.

<sup>5</sup> *Ibid.*, §20.

<sup>6</sup> *Fund. Math.*, 27, 242 (1936).

<sup>7</sup> *Topology* (1930), 287.

<sup>8</sup> Compare Vedenisoff, *Fund. Math.*, 27, 234-238 (1936).



# INTEGRATION AND DIFFERENTIATION IN PARTIALLY ORDERED SPACES

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We consider a vector space  $S$  which is partially ordered (relation  $>$ ). By definition,  $S$  is an Abelian group of addition with the ring of real numbers as coefficients (operators), and corresponding to any two elements  $a, b$  of  $S$  there exists a unique element  $\sup(a, b)$  and a unique element  $\inf(a, b)$ . As usual,  $\sup(a, 0) - \inf(a, 0)$  will be denoted by  $|a|$ . We assume that  $S$  is complete: every set of elements  $A$  which is bounded from above (below) has a  $\sup$  ( $\inf$ ). This leads to a sequential limit topology in  $S$ . Namely,  $a_n \rightarrow 0$  if

$$\inf_n \sup(|a_n|, |a_n + 1|, \dots) = 0. \quad (1)$$

In this connection we introduce elements  $+\infty$  and  $-\infty$  with properties stated by L. V. Kantorovitch.<sup>1</sup> A concept of limit which requires more than (1) is dominated convergence; it requires the existence of an element  $a_0 > 0$  such that  $|a_n| \leq \epsilon a_0$  for  $n \geq N(\epsilon)$ ,  $\epsilon$  real. However, Kantorovitch has shown that convergence is equivalent with dominated convergence if the following axiom is fulfilled.

*Axiom (K).* If  $A_m$  ( $m = 1, 2, \dots$ ) is a set of elements of  $S$  which is bounded from above then there exists a finite subset  $A'_m$  such that

$$\lim_{m \rightarrow \infty} \sup A'_m = \lim_{m \rightarrow \infty} \sup A_m$$

if the limit on the right hand exists.

We will later add another axiom. However, we will not make the assumption that our space has a Banach norm. For the construction of a Lebesgue theory of integration the Banach norm can be replaced by axiom (K). We consider a countably additive Boolean algebra of sets  $E$  of an arbitrary space  $B$  and a countably additive numerical measure  $\mu E$  on it, and we make the assumption that  $B$  is also measurable and that  $\mu B$  is finite. We call a sequence of functions  $f_n(t)$  from  $E$  to  $S$  uniformly convergent to  $f(t)$  if there exists a sequence of element  $a_n \rightarrow 0$  such that  $|f_n(t) - f(t)| < a_n$ , and we call it *convergent in measure* if for any numerical  $\epsilon > 0$  there exists a subset  $E_0$  of  $E$  such that  $\mu(E - E_0) < \epsilon$  and  $f_n(t)$  converges uniformly on  $E_0$ . A step function  $s(t)$  on  $E$  is one which has a constant value  $a_r$  on each set  $E_r$  of a finite partition of  $E$ , and the integral  $\int s(t) d\mu$  on  $E$  is the sum  $\sum a_r \mu E_r$ . We now call a function  $f(t)$  measurable if it is the limit in measure of a sequence of step functions  $s_n(t)$ ; we call it integrable if the integral of the double sequence  $|s_m - s_n|$  tends to 0; and we



define the integral of  $f(t)$  as the limit of the integral of  $s_n(t)$ . At this juncture axiom (K) plays its rôle: a limit in measure of measurable functions is again measurable; the value of  $\int f(t)d\mu$  is independent of the approximating sequence  $s_n(t)$ ; furthermore, if a measurable function is dominated by an integrable function, it is also integrable and if a dominated sequence converges in measure then the integral of the limit is the limit of the integral. It is important to note that convergence in measure implies convergence almost everywhere and that we are making no statement about the converse.

An additive set function  $F(E)$  from  $B$  to  $S$  is absolutely continuous if there exists an error function  $e(\eta)$  from real numbers to  $S$ , which tends to 0 with  $\eta$  such that  $|F(E)| \leq e(\mu E)$ . The indefinite integral of an integrable function is absolutely continuous, however the converse is not true. In the case of Banach spaces satisfactory criteria for  $S$  are known under which the converse is valid.<sup>2</sup> As for our partially ordered spaces, we will not give a complete discussion but state one criterion for sufficiency. We will say that  $S$  has the property (D) if every absolutely continuous function is an indefinite integral.  $S$  having the property (D) does not depend on the Boolean algebra provided no set of positive measure is indivisible; and it is equivalent with the property of every monotone function  $f(t)$  from the real interval  $0 \leq t \leq 1$  to  $S$  having a finite derivative almost everywhere. From this equivalence it can be seen that property (D) is implied by the following axiom, which for instance is fulfilled for the ordinary  $L_p$  spaces,  $p > 1$ .

*Axiom (L).* There exists on  $S$  a countable number of numerical additive functionals  $\alpha_n = L_n(a)$ , each of which is non-negative for positive  $a$  and continuous in the sequential topology of  $S$ , such that  $L_n(a) > 0$  for all  $n$  implies  $a > 0$  and that, for  $a_1 \leq a_2 \leq \dots$ ,  $\lim_{m \rightarrow \infty} L_n(a_m) < +\infty$  for all  $n$  implies  $\sup a_m < +\infty$ .

Axiom (L) allows us to generalize many other theorems none of which have been known for Banach spaces. The most interesting is Birkhoff's *strong ergodic theorem*: if  $f(t)$  is an integrable function with period 1 from real numbers to  $S$  then

$$\frac{1}{n} \sum_{\nu=1}^n f\left(t + \frac{\nu}{n}\right)$$

has a limit as  $n \rightarrow \infty$  for almost all  $t$ . Furthermore if

$$\sum (a_n \cos 2\pi nt + b_n \sin 2\pi nt)$$

is the Fourier series of  $f(t)$  and  $f(t)$  satisfies a Lipschitz condition of order  $> 1/2$  (in particular if  $f(t)$  has a bounded derivative) then

$$\sum (|a_n| + |b_n|) < +\infty. \quad (2)$$

Finally, if  $f(t)$  is an almost periodic function and if all exponents  $\lambda_n$  which occur in its Fourier series

$$\sum (a_n \cos \lambda_n t + b_n \sin \lambda_n t)$$

are linearly independent then (2) holds again.

<sup>1</sup> "Lineare halb-geordnete Raume," *Recueil mathématique, Moscou*, 2, 121-168 (1937).

<sup>2</sup> For the literature see B. J. Pettis, "Differentiation in Banach spaces," *Duke Jour.*, 5 (1939).

<sup>3</sup> A. Zygmund, *Trigonometrical series*, p. 135.

## GALACTIC AND EXTRAGALACTIC STUDIES, III. PHOTOGRAPHS OF THIRTY SOUTHERN NEBULAE AND CLUSTERS

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Approximately three-fourths of the external galaxies that are near enough for close classification are spirals, and most of the spirals can be easily placed in half a dozen common categories. Similarly the spheroidal galaxies are readily assigned to a few subtypes, rarely with abnormalities among them. The unusual forms, whether chaotically irregular like the Magellanic Clouds, or merely peculiar variations on the usual types of spiral and spheroidal galaxy, are, however, of uncommon interest and perhaps of special importance in the study of galactic structures and development.

Several instructive curiosities and abnormal forms are included among the nebulae and clusters represented on the following pages. Some have already been published by Harvard, or by other observatories, but most of them are here presented for the first time. With the exception of the reproduction in figure 2, all photographs were made with the 60-inch reflector at the Boyden Station, Bloemfontein. The scale is 26" to the millimeter. Generally we have used Cramer High Speed emulsions, developing with rodinal. The reproduced photographs are selected from plates made in the regular course of work on clusters and nebulae; no particular pains were taken to obtain photographs specially suited to publication. As usual, the finer details are lost in the reproduction, but enough remains to indicate the peculiar importance of some of these objects and the advisability of further study, photometric and spectroscopic. In all figures North is at the top, West on the right, except for figure 1 for which West is at the top and South on the right.

In table 1 we have listed the principal objects of each photograph, giving coördinates and approximate magnification. The brief tabulated descriptions are amplified in the following comments on the individual figures.

1. The remarkable assembly of clusters and of bright and dark nebulosities that make up the Eta Carinae Nebula is too large for full exhibition by a single 60-inch reflector plate. The diameter is about  $2^{\circ}.5$ , and therefore less than a fifth of the area illuminated

TABLE 1  
LIST OF REPRODUCED CLUSTERS AND NEBULAE

FIGURE	NGC	$\delta$	R. A. $m$	(1900) DEC.	GALACTIC LONG.	LAT.	TYPE	ENLARGEMENT
1	$\eta$ Car	10	41.2	-59 09	255	- 1	Gaseous nebula	2.0
2	6514	17	56.3	-23 02	335	- 2	Trifid nebula	0.9
	6523	17	57.6	-24 23	334	- 3	Lagoon nebula	0.9
	6531	17	58.6	-22 30	335	- 2	Open cluster	0.9
3	7793	23	52.7	-33 07	330	-79	Spiral	1.5
4	5236	13	31.4	-29 21	283	+31	Spiral	1.0
5	7582	23	12.9	-42 54	314	-67	Spiral	1.0
	7590	23	13.4	-42 47	314	-67	Spiral	1.0
	7599	23	13.8	-42 48	314	-67	Spiral	1.0
6	1097	2	42.1	-30 41	193	-64	Barred spiral	1.5
7	55	0	10.0	-39 46	296	-77		1.0
8	5189	13	26.4	-65 28	275	- 4	Gaseous nebula	10.0
9	2442-3	7	36.6	-69 18	248	-21	Barred spiral	1.6
10	1566	4	17.8	-55 11	231	-43	Spiral	1.0
11	6753	19	03.0	-57 12	307	-26	Spiral	3.0
12	1559	4	16.4	-63 02	241	-41	Barred spiral?	1.5
13	6935	20	31.0	-52 27	314	-39	Plate spiral	1.5
	6937	20	31.4	-52 30	314	-39	Plate spiral	1.5
14	4782	12	49.3	-12 02	273	+50	Double spheroidal	2.4
	4783	12	49.4	-12 03	273	+50		
15	2736	8	56.9	-45 30	235	+ 1	Streak nebula	1.0
16	3581	11	07.7	-60 46	259	- 1	Gaseous nebula	1.6
17	3132	10	02.8	-39 57	240	+13	Planetary	3.4
18	I 4602	17	37.2	-64 38	296	-19	Magellanic type	1.5
19	6438	17	52.9	-85 25	275	-27	Spheroidal	3.0
		17	53.4	-85 25	275	-27	Magellanic type	3.0
20	4038-9	11	56.8	-18 19	256	+43	Ring-tail spiral	2.6
21	5139	13	20.8	-46 57	277	+14	Globular cluster	1.5
22	7099	21	34.7	-23 38	355	-48	Globular cluster	1.5
23	2818	9	12.0	-36 12	230	+ 9	Gaseous nebula in open cluster	1.5

by bright nebulosity is shown in figure 1. A smaller scale photograph of the whole region, with the 24-inch Bruce reflector of the Boyden Station, is shown on the cover of *The Telescope* for May-June (1937). The section of the nebula here shown is the brightest part, and is most interestingly marked with sharp-edged dark streaks. The eighth magnitude star, Eta Carinae, which behaved like a nova a century ago and is now possessed of a peculiar bright-line spectrum, is located 2.4 cm. below the center of the photograph. On a red-sensitive plate this star is relatively conspicuous, but otherwise no

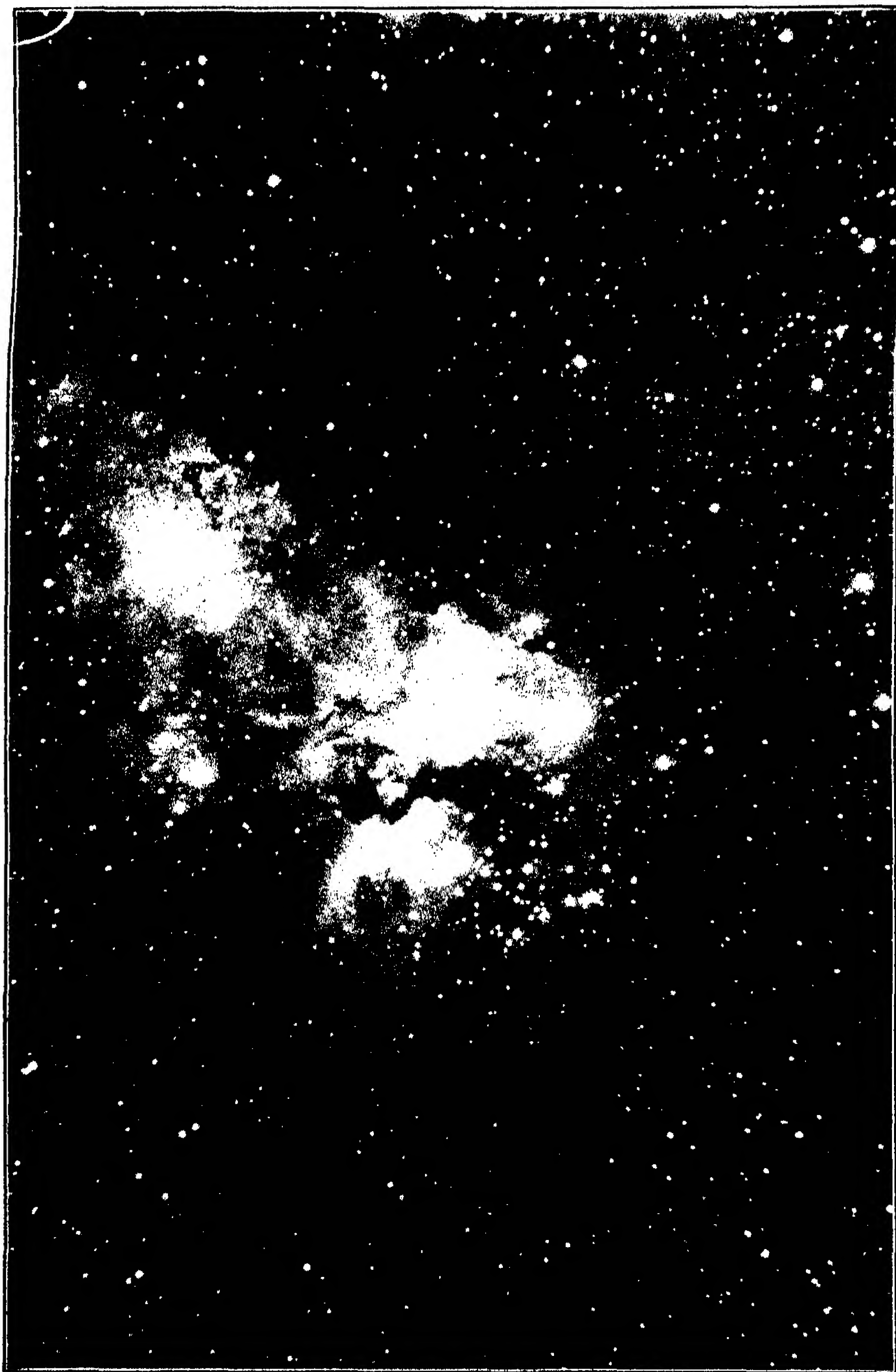


FIGURE 1



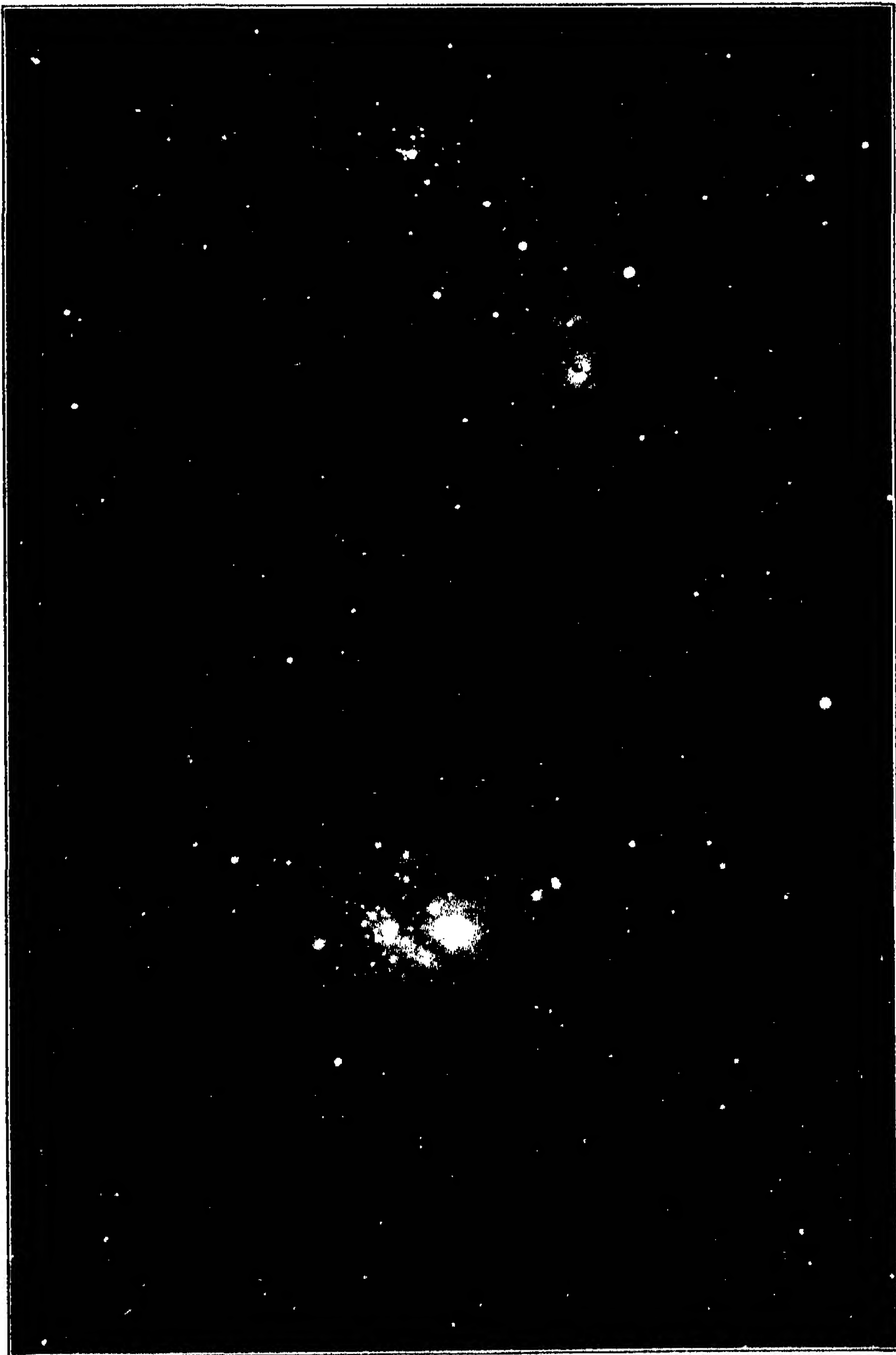


FIGURE 2





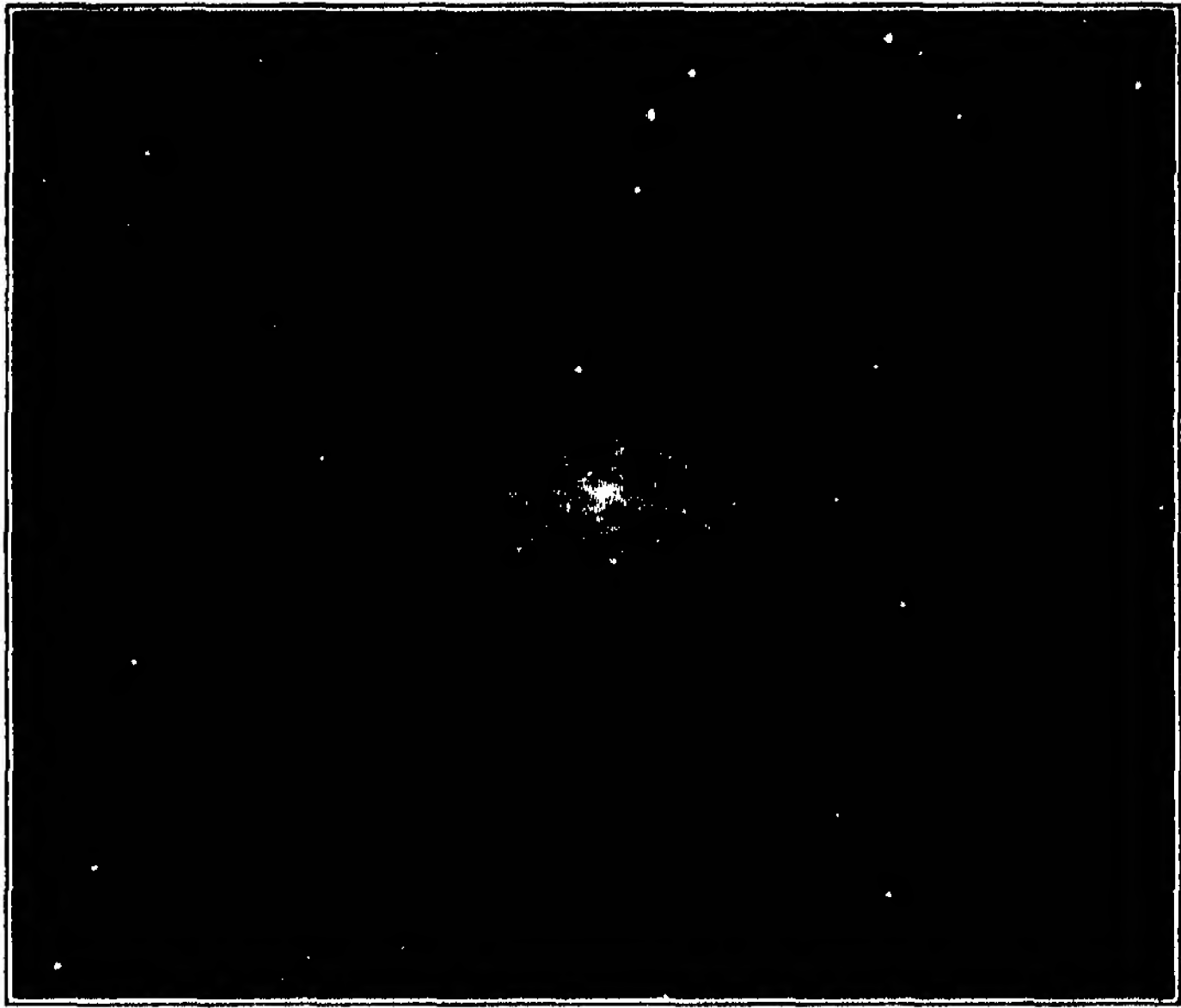


FIGURE 3



FIGURE 4





FIGURE 5

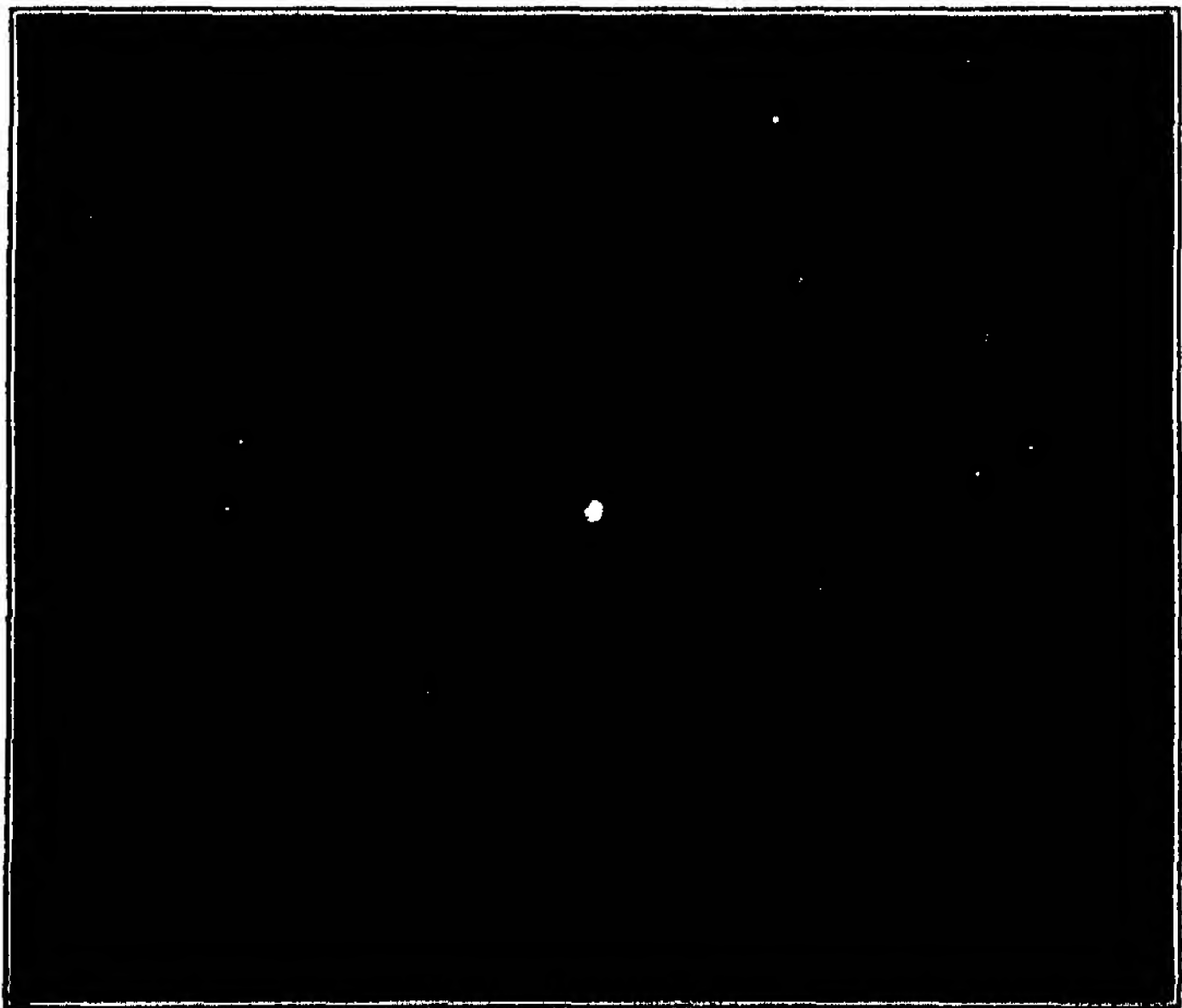


FIGURE 6



FIGURE 8

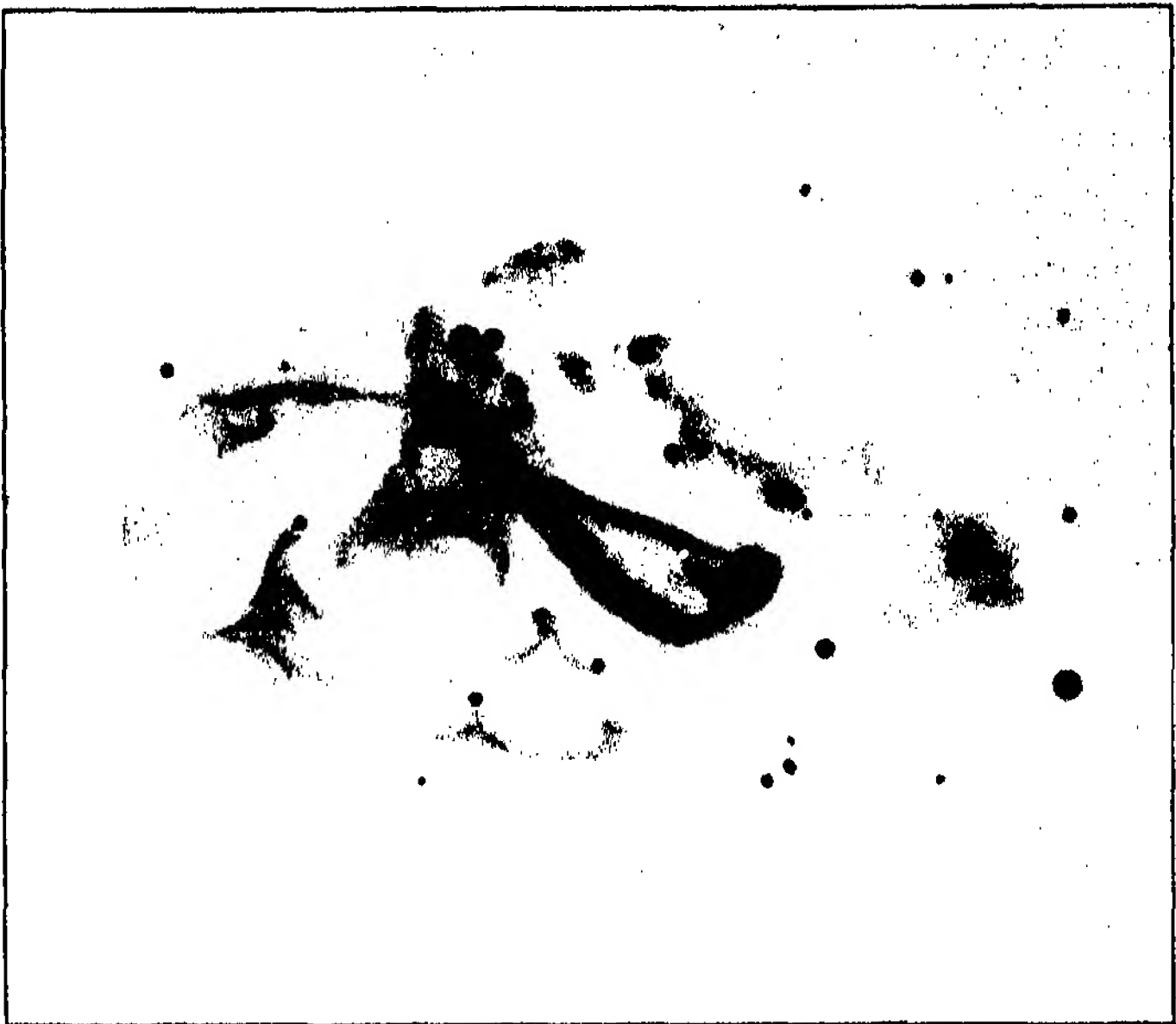


FIGURE 7







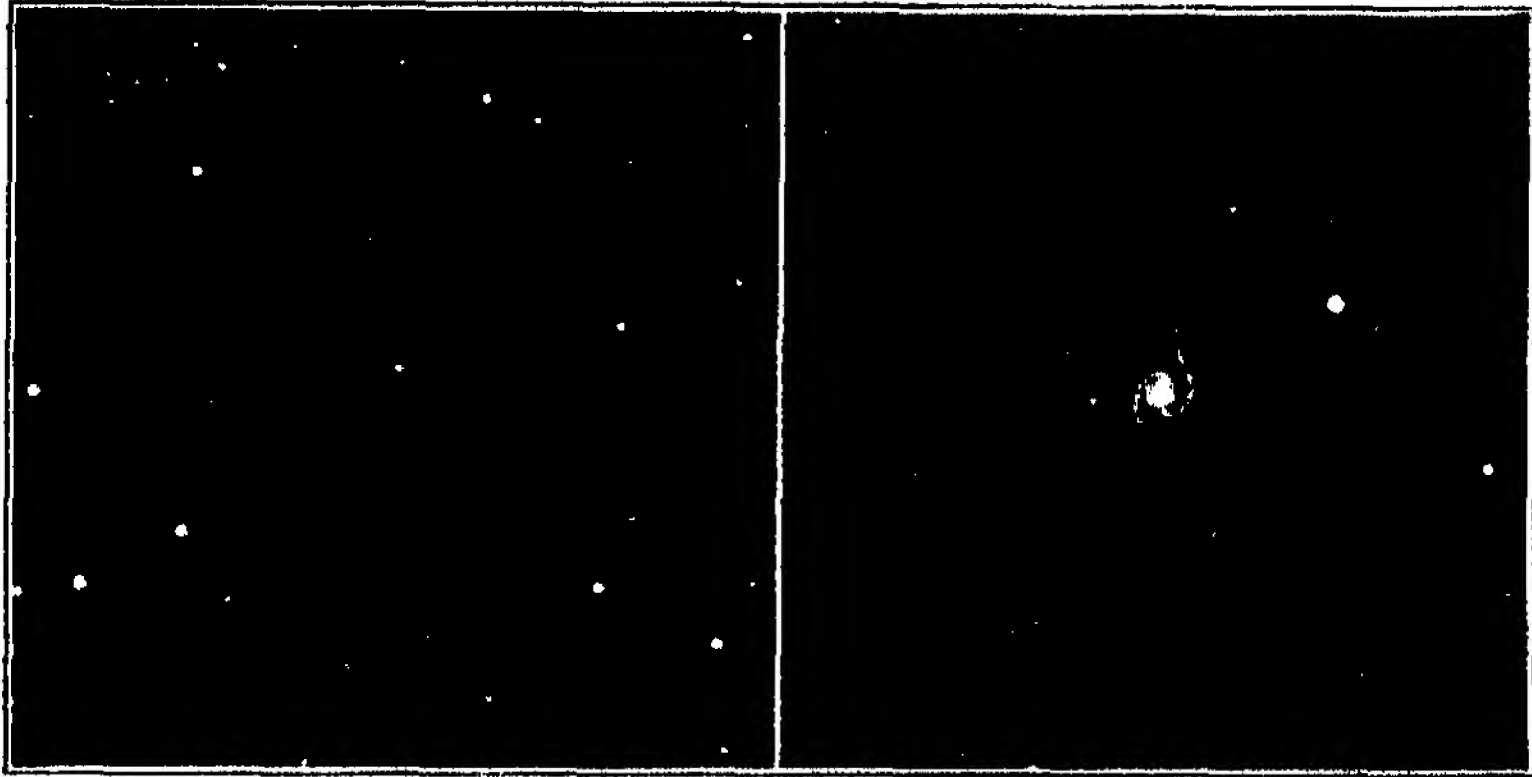


FIGURE 9

FIGURE 10

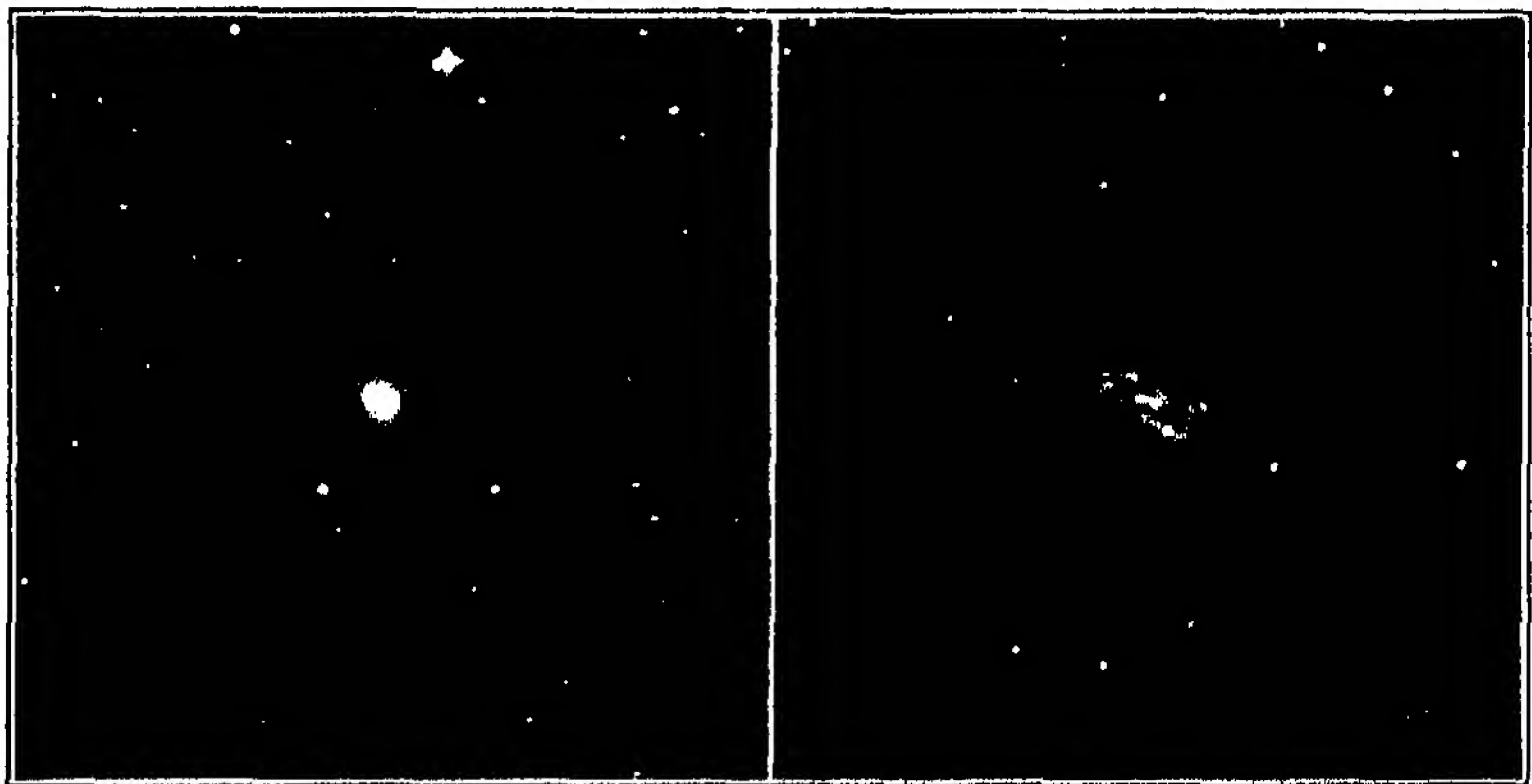


FIGURE 11

FIGURE 12

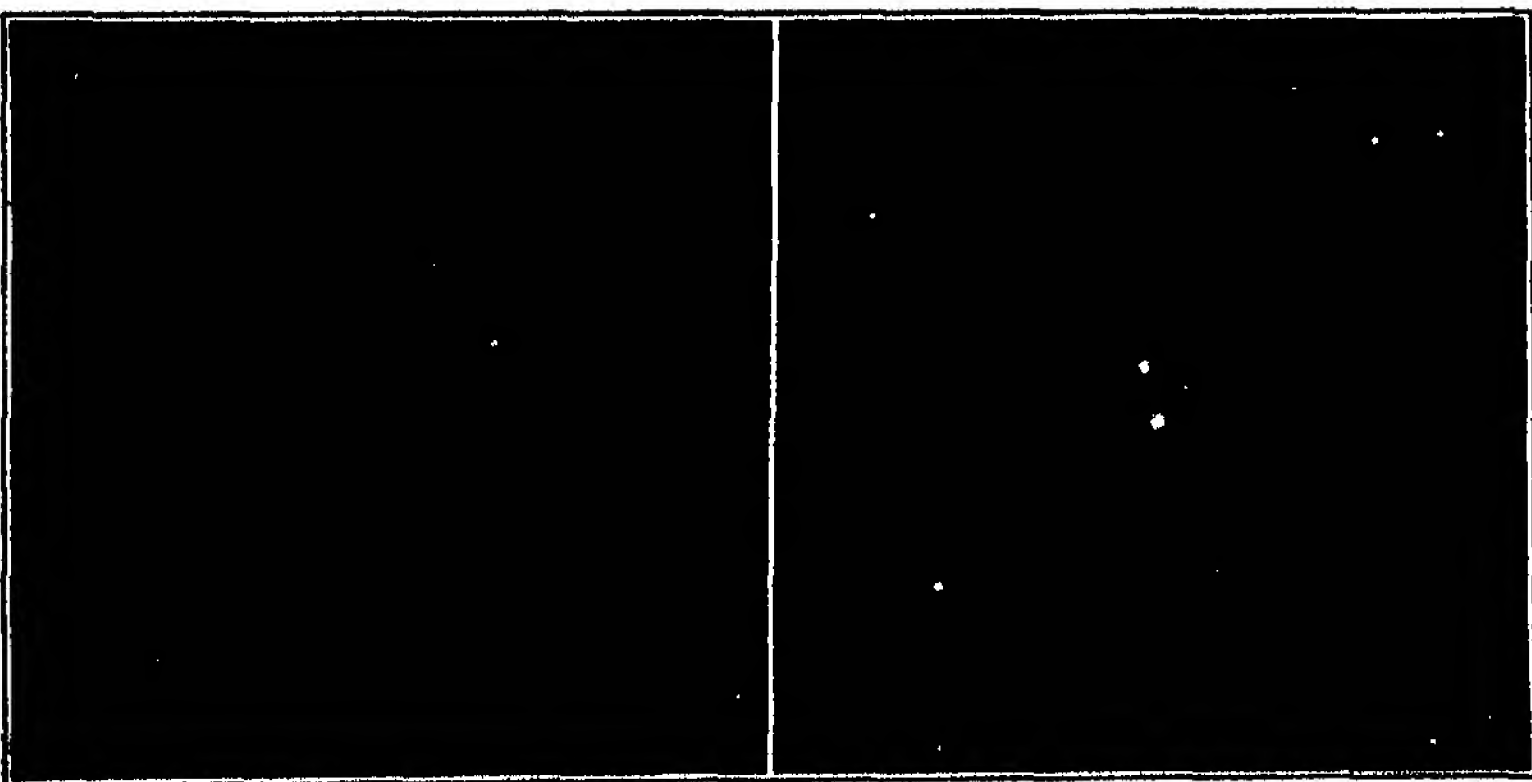


FIGURE 13

FIGURE 14



FIGURE 19



FIGURE 20



FIGURE 17



FIGURE 18



FIGURE 15

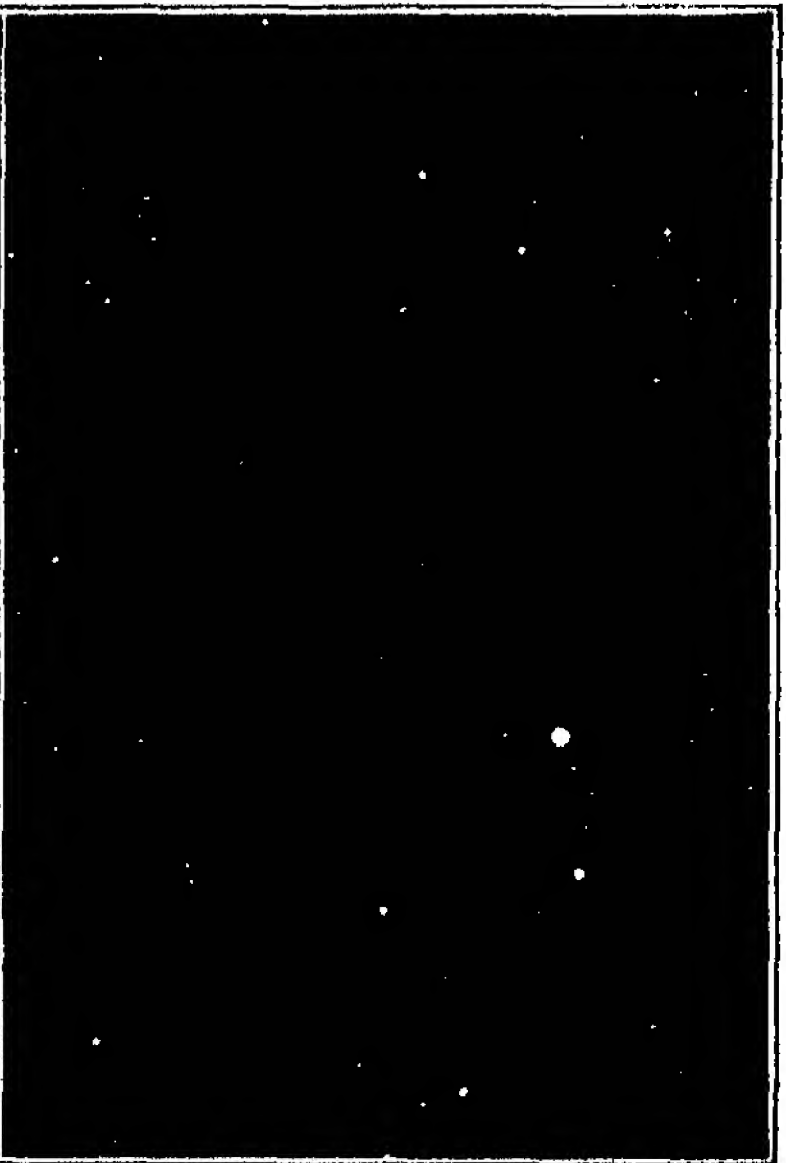


FIGURE 16





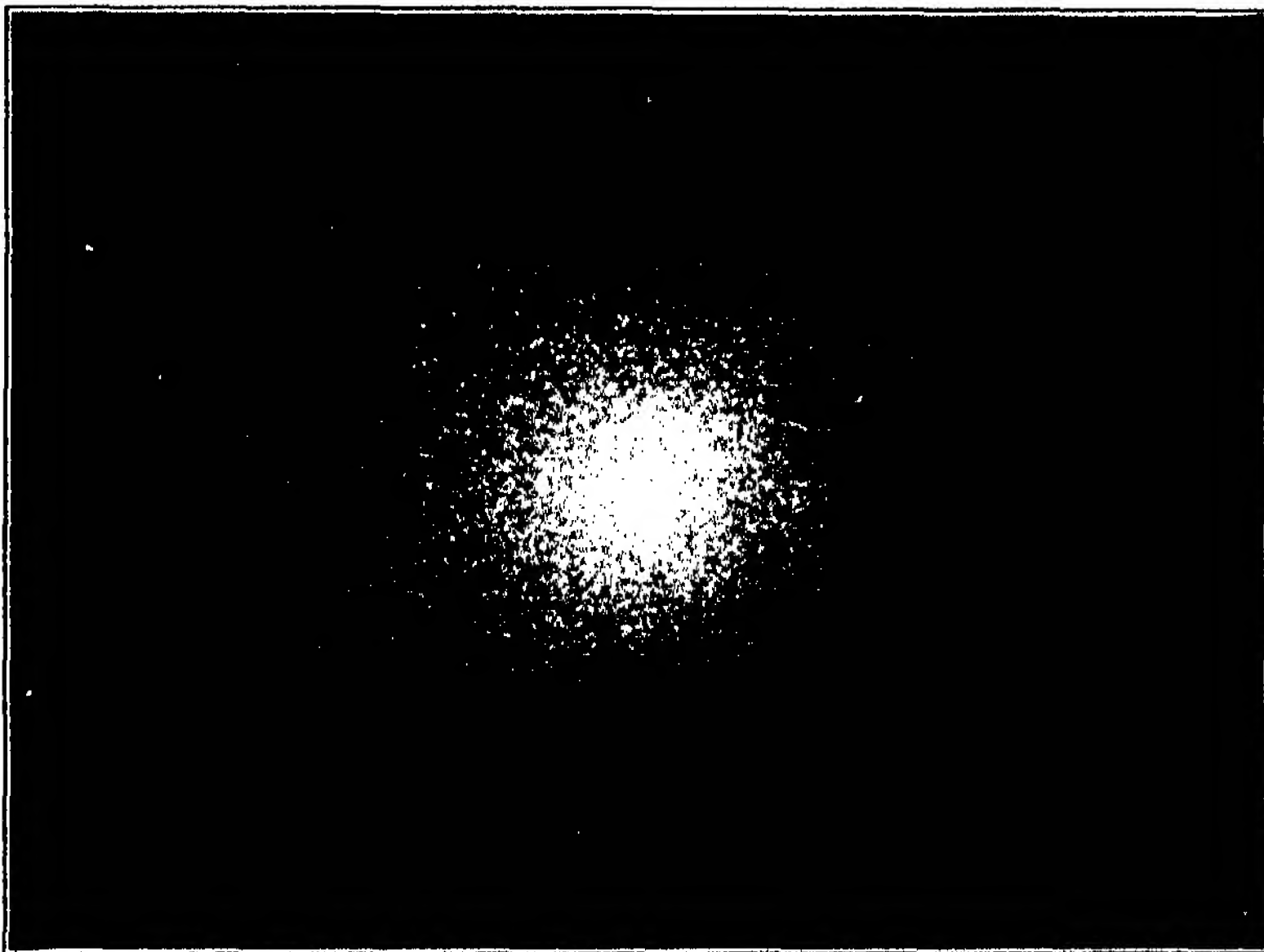


FIGURE 21

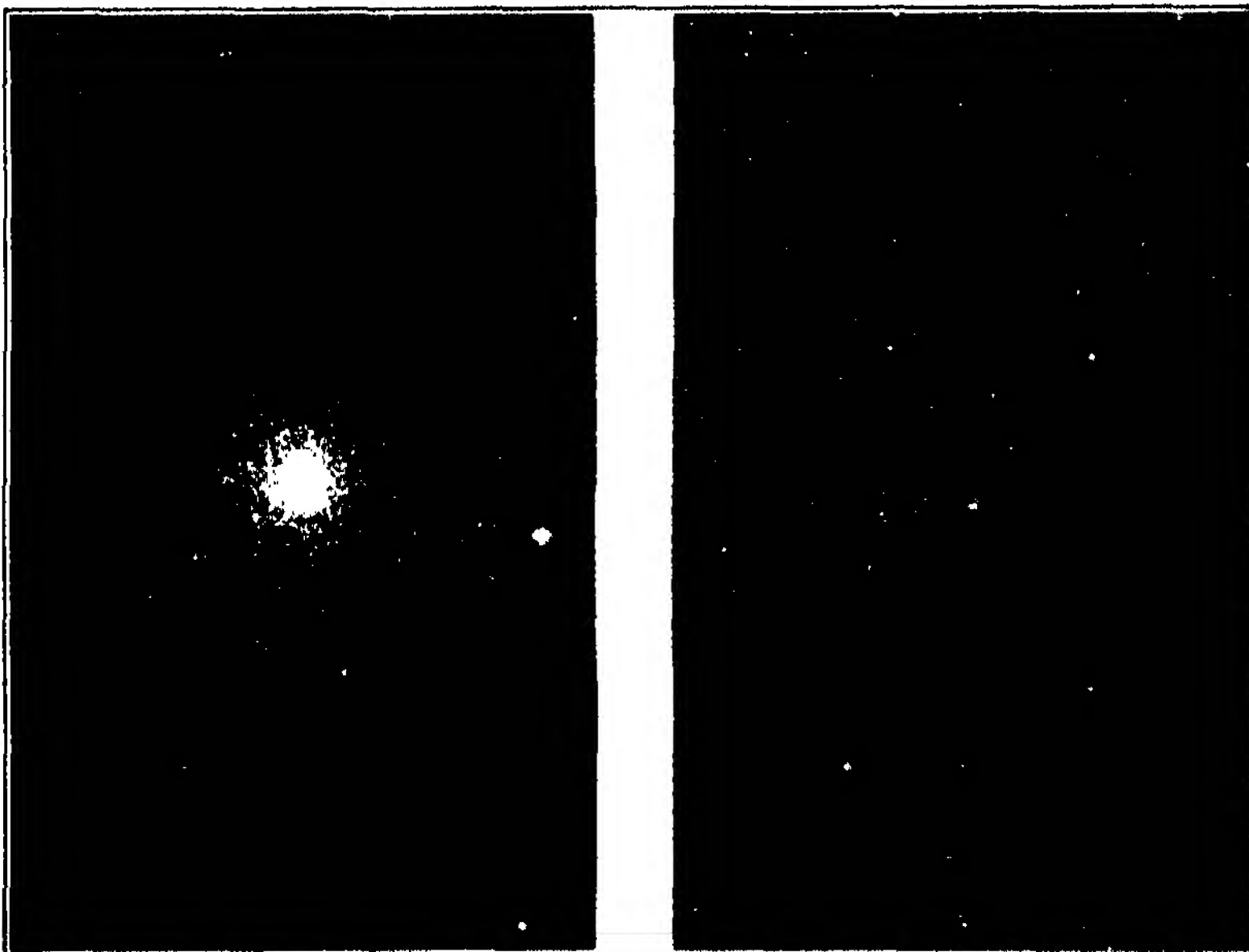


FIGURE 22

FIGURE 23





striking differences between red and blue plates are to be noted, except the sharper contrast on the red plate between light and dark nebulosity along the borders and in the crater-like area, 1.3 cm. in diameter, 1.6 cm. above and to the left of Eta Carinae. Star clusters stand out more sharply on the red plates. The reproduced blue photograph had an exposure of one hour, with the seeing "unsteady and fuzzy at times."

2. The four-hour exposure with the Bruce doublet on a region in Sagittarius ten degrees from the galactic center shows both the strong irregular absorption that marks this interesting section of the Milky Way and two familiar objects: the Lagoon Nebula, Messier 8 (NGC 6523) with its star cluster, and the Trifid Nebula, Messier 20 (NGC 6514). Also, at the center top of the picture is the bright open cluster Messier 21 (NGC 6531). That these two large gaseous nebulae are separated by scarcely more than the diameter of Messier 8 is commonly overlooked. Stars fainter than the eighteenth magnitude appear on the reproduction. The area shown is approximately  $2^{\circ}.3$  by  $3^{\circ}.4$ .

3. The nebular class *Sd* might well be used for NGC 7793 and similar objects of the spiral class that go far beyond the average *Sc* type in openness and lack of central concentration. Spiral arms are not distinct for NGC 7793; for the typical *Sc* spiral Messier 83 in figure 4 they dominate the structure.

A photometric study of NGC 7793 by Shapley and Mohr is published in *Harvard Bulletin* 907 (1938). The object was discovered in 1850 during cometary searches by George P. Bond, working visually with a comet seeker at the Harvard Observatory. A noteworthy feature, shown in figure 3, is the group of outlying patches of irregular nebulosity, especially at the western central edge of the photograph and in the southwestern quadrant. The reality of these nebulous patches is verified by other photographs. They are probably distantly outlying parts of the spiral nebula, although apparently far beyond detectable spiral arms. Since the distance of the spiral is estimated at 810 kpc., the diameters of the patches are of the order of 100 pc. Probably a similar outlying patch near the spiral Messier 101 is the irregular object NGC 5477; but NGC 5477 has a nucleus, whereas many of the nebulosities near NGC 7793 are without central concentration. Exposure 68 minutes.

4. NGC 5236 = Messier 83 is one of the brightest spirals in the sky (see plate II, *Lick Obs. Pub.*, 13, 42 (1918)). It lies in a region richly populated with faint nebulae, and is within two degrees of the great Centaurus cluster of galaxies (*Harv. Bull.* 874 (1930)). Exposure 75 minutes, with the seeing recorded as "impossible."

5. The spirals NGC 7582-90-99, which probably form a physical system, are much alike in dimensions, inclination and class, but they differ considerably in structural details. Faint spiral arms appear to envelop the main body of NGC 7582; and NGC 7599 has no strong nucleus. Within two degrees of these three are five other equally large and bright spirals, NGC 7496, 7531, 7552, 7632 and IC 5325—all probably members of the group. The reflector plate of two hours exposure, reproduced in figure 5, shows 154 faint nebulae, many of which are retained in the reproduction.

6. The unusual barred spiral NGC 1097 loses much of its fine structure in the reproduction (see also Reynolds, *M. N.*, 85, 101 (1925)). The large nucleus (burned out in the reproduction) shows a strong rift and a peculiar internal structure that perhaps result chiefly from the distribution of obscuration. Along the swollen or elliptical "bar" and in the spiral arms there are also peculiar obscurations and markings.

The spiral of class *Sa*, 17 mm. to the northwest of the nucleus and apparently within one of the spiral arms, is probably not connected with NGC 1097; its magnitude (on small-scale plates) is approximately 14. Exposure 101 minutes.

7. With a diameter of more than half a degree ( $32'.6 \times 4'.4$ ), NGC 55 probably is exceeded in angular dimensions among the spirals only by the Andromeda Nebula and Messier 33. Since it is considerably more distant than either of these objects, its linear

dimensions also may be outstanding among the galaxies. The object is so peculiar that it cannot easily be classified. Is it, perhaps, a highly resolved spiral without nucleus, nearly on edge; or an equally tilted system of the Magellanic type; or possibly two overlapping systems, similarly inclined, with similar internal structure? The third interpretation, suggested in conversation by Dr. G. Z. Dimitroff, is lent color by the appearance of the object on small-scale long-exposure plates (see inset in figure 7), where it appears to be a double, edge-on spiral, without nuclei and with the components differing perhaps three magnitudes in total brightness. Strong absorption may conceal nuclear regions; but a number of spirals that appear to be devoid of a central nucleus or strong central concentration are now known. It may well be that such objects will need to be recognized as forming a distinct class.

No Cepheid variable stars or novae have as yet been found in NGC 55, but South African plates for the detection of such distance indicators are accumulating. The brightest stars are of the eighteenth magnitude, and we might conclude that the system is of the order of 0.7 mpc. distant with an overall diameter of 7 kpc. Exposure two hours; poor seeing.

8. NGC 5189, near the galactic equator, is a gaseous nebula of such remarkable knotted structure that it is here best represented by a drawing, made by Miss Virginia McKibben from an original reflector plate. No bright star is involved in the nebulosity, but 6' to the south is the seventh magnitude star H. D. 117694, spectrum B9. In the Henry Draper Catalogue there is no star with a spectrum earlier than B5 within a degree of the nebulosity. The overall dimensions of the nebula are  $3'.0 \times 2'.0$ . Exposure two hours.

9. The barred spiral NGC 2442-3 very closely resembles NGC 1097 (figure 6, above) in angular dimensions and structure, but it is more than a magnitude fainter and its ellipsoidal and structureless nucleus is decidedly off center. The low latitude,  $-21^\circ$ , probably accounts for the difference in brightness, and is a measure of the space absorption. The dark absorbing band that follows along the middle of the northern arm is unusually conspicuous. (The large marking across the end of this arm is a plate defect.) Exposure 90 minutes.

10. NGC 1566 is a tenth-magnitude spiral conspicuous for the series of bright clusters, nebulosities or supergiant stars lined up along both spiral arms. Less than one turn from the nucleus the arms suddenly become completely devoid of these condensations. Exposure two hours.

11. NGC 6753 is a face-on spiral; its interesting nuclear structure is not shown clearly in the reproduction. The thin closely wound arms start from the rim of a sharp-edged nearly circular disc,  $23''$  in diameter, much as with a typical barred spiral, but there is no certain indication of the typical bar. The bright nucleus in the center of the disc is nearly stellar in sharpness. See the description below of figure 13. Exposure one hour; seeing fair.

12. The high latitude,  $-41^\circ$ , of NGC 1559 probably makes it necessary to conclude that the object is really a galaxy of peculiar form, perhaps an irregular barred spiral. The most central of the twenty nebulous condensations is not the brightest. There is too much regular structure to justify assigning this object to the Magellanic type. When the west side is taken as the top of the picture, the form of the object suggests a giraffe's head. Exposure one hour; seeing unsteady.

13. It is remarkable that two such unusual but similar systems as NGC 6935 and 6937 should be only 4' apart, and both face-on. In total brightness, in diameter of the central plate or disc and in magnitude of the central nucleus they are almost identical; but NGC 6937 shows distinct spiral arms, and its companion only faintly suggests an external structure of some sort just outside the bright-rimmed plate. Densitometer

tracings verify the appearance in detail, showing for both objects that the rim of the nearly circular plate is brighter than the plate itself, and showing also for NGC 6935 the probable existence of spiral arms. For NGC 6937 there is a suggestion of a bar across the center; probably these "plate spirals" are related to the common barred spirals. The nuclear structure of NGC 6753 (figure 11, above) is similar. An examination of many other nebular plates taken with the Bruce and Metcalf telescopes shows that NGC 4622 and NGC 5055 (Messier 63) may be of this same type, and NGC 4507 and NGC 4750 are somewhat similar. Perrine has described NGC 6935-37 in *Monthly Notices*, **82**, 487 (1922). Exposure one hour.

14. NGC 4782-3 appear on first inspection to be a connected pair of spheroidal nebulae, but the densitometer tracings show that one of the objects (NGC 4782) has a structure suggestive of a class  $S0^*$  or  $Sa$  spiral. The bridge between the two objects may therefore be illusory. Many plates show the apparent connection distinctly. Exposure one hour.

15. The brightest star in the picture of NGC 2736, the "Streak" nebula, is H. D. 77433, spectrum A3. This peculiar streak of nebulosity, 20' long, appears wedge-shaped, on the original plate, about 3' wide at the base, with the point of the wedge to the south; but only the bright western boundary of the nebulous area shows clearly in the reproduction. Exposure one hour; seeing poor.

16. The diffuse gaseous nebulosity in Carina, NGC 3581, is on the galactic equator, less than  $4^\circ$  east of the Eta Carinae Nebula. The reproduction satisfactorily brings out the details of bright and dark nebulosity as shown by the original plate, although by increasing the contrast the interesting indistinct obscurations at the top of the picture could be enhanced. Exposure 75 minutes; "at times through thin haze."

17. A series of photographs of varying exposures would be necessary to bring out the intricate details of the bright planetary NGC 3132 = H. D. 87877. It could well be named the "8-burst" planetary from the number of distinct arcs on the boundary of the main disc or shell. The class A star H. D. 87892, magnitude 9.5, is centrally superposed. The object has been studied spectroscopically by the Lick Observatory (*Lick Obs. Pub.*, **13**, 118 (1918)). Exposure one hour.

18. A new object of the Magellanic Cloud type is revealed by the photograph of IC 4662. The next figure also shows an object of this kind, but neither is bright enough to be considered a member of the local group of galaxies. IC 4662 is resolved on the 60-inch plates, but its brightest stars are of about the nineteenth magnitude, with star clusters up to the fifteenth magnitude or brighter. Exposure 99 minutes; seeing fair.

19. NGC 6438, described in Dreyer's catalogue as "pretty bright, round, very gradually brighter in middle," refers only to the spheroidal member of a strange pair—probably a physical double. The other member is of the Magellanic type, unresolved on the 60-inch plates. The moderately high latitude,  $-27^\circ$ , much decreases the likelihood that the irregular component is a gaseous nebula, superposed on the field. There is no neighboring high temperature star. The affiliation of these two extreme forms of galaxies reminds one of the analogous association of the giant globular cluster 47 Tucanae and the Small Magellanic Cloud, but that association is a coincidence of direction, since the Small Cloud is perhaps five times the distance of the cluster. Exposure two hours.

20. Two catalogue numbers, NGC 4038 and NGC 4039, have been given to the very peculiar "ring-tail" structure shown in figure 20. The object has been described by Gregory, Perrine and Duncan, and a picture made with the Mount Wilson 100-inch reflector is given in *Mount Wilson Contribution* 256 (1923). The high latitude,  $+43^\circ$ , gives assurance that the object belongs to the category of external galaxies. It is reminiscent of the "giraffe's head" in figure 12 above, but is much more like NGC 4027 (not reproduced here). The two are, in fact, nearly identical, and again we note as remark-

able that such odd structures come in pairs; they are separated in the sky by only seven-tenths of a degree, and are of nearly the same magnitude (11.0 and 11.6), angular dimensions, and structural peculiarities. Almost certainly they are physically associated, or have been. (The same must also be true for the "plate spirals" in figure 13.) The objects are one-armed spirals of a sort, with open ring nuclei. At least for NGC 4038-9, a hazy irregular nebulosity, cut by absorbing lanes, surrounds the ring and arm. The Bruce plates clearly show the great extent of two streamers from this hazy enveloping nebulosity, giving an overall diameter of more than a quarter of a degree (*Mt. W. Contr.* 256, 4 (1923); *Harv. Ann.*, 88, 98 (1934)). The central ring and a part of the arm are outlined by bright stars or stellar groups.

A spectroscopic study of the object will be undertaken by the McDonald Observatory; a photometric analysis will be made at Harvard. Exposure one hour.

21. The globular cluster Omega Centauri = NGC 5139, shown here with a sixty-minute exposure under fairly good conditions of seeing, is unquestionably one of the most remarkable stellar systems. Its total absolute magnitude is comparable to that of the smaller external galaxies, such as the companions to the Andromeda Nebula.

22. NGC 7099 is the globular cluster Messier 30.

23. An open galactic cluster in low latitude and a small bright gaseous nebula together bear the catalogue number NGC 2818. The star cluster is little more than a chance thickening of the galactic star field.

\* A class between *E7* and *Sa*, recently proposed by Hubble.

## ON THE DETERMINATION OF THE ORBITAL ELEMENTS OF ECCENTRIC ECLIPSING BINARIES

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A systematic and elegant procedure was devised<sup>1, 2, 3, 4</sup> by H. N. Russell and H. Shapley, for finding the orbital elements of eclipsing stars from their light curves. In their procedure the fundamental equations for the loss\* of light,  $\alpha$ , during an eclipse in a circular orbit, are

$$\cos^2 i + \sin^2 i \sin^2 \theta = \delta^2 \quad (1)$$

and

$$\delta = r_1 \{1 + kp(k, \alpha)\}, \quad (2)$$

where  $i$  is the inclination,  $\theta$  is the mean anomaly measured from mid-eclipse,  $\delta$  is the apparent distance between centers,  $r_1$  is the radius of the larger star,  $kr_1$  is the radius of the smaller star, the unit of distance is the radius of the relative orbit, and  $p(k, \alpha)$  is a known and tabulated function



of  $k$  and  $\alpha$ . If the orbit is eccentric, then equation (1) must be replaced by equation (23) of Russell's<sup>2</sup> paper II; this exact equation

$$r^2 \{ \cos^2 i + \sin^2 i \cos^2 (v + \omega) \} = \delta^2 \quad (23)$$

may be written in the equivalent form

$$\{ \cos^2 i + \sin^2 i \sin^2 (v + \omega - 90^\circ) \} \frac{(1 - e^2)^2}{(1 + e \cos v)^2} = r_1^2 [1 + kp(k, \alpha)]^2. \quad (23')$$

In the last two equations the unit of distance is the semi-major axis of the orbit of the brighter star relative to the fainter,  $r$  is the radius vector,  $e$  is the orbital eccentricity,  $v$  is the true anomaly, and  $\omega$  is the longitude of periastron measured from the ascending node in the direction of motion. The problem that arises in practice is to find a set of elements that, inserted in equation (23'), will reproduce to within the observational accuracy the known, rectified, light curve. Such definitive elements can be obtained in general only by the adjustment of preliminary, approximate, values. By treating each eclipse-curve separately as though the orbit were circular, "circular" elements can be obtained from either or both of them, and Russell<sup>2</sup> gave simple equations for finding, from the "circular" elements, approximate values of the true elements. Russell's equations yield closely approximate elements when  $e$  is small; but the equations are correct only through terms of the first degree in  $e$ , and when  $e$  exceeds two or three tenths their accuracy is impaired.

For most of the known eclipsing stars,  $e$  is smaller than two tenths; but some eclipsing stars are known for which  $e$  considerably exceeds<sup>†</sup> this limit. Precise orbital elements of such stars are needed in order to investigate their concentrations<sup>6, 7, 8</sup> of density; and it therefore becomes desirable to obtain formulae, closely approximate for large values of  $e$ , that express the true elements in terms of the "circular" ones. In this note, such formulae are presented. Several alternative forms for them are possible, differing from one another in accuracy and complexity. The more accurate forms are in general the more complicated. It is desirable for the formulae to be simple and easy of application; and since the resulting approximate values of the true orbital elements should probably always be tested and if necessary improved by the exact equation (23'), it is permissible to sacrifice some accuracy in order to gain simplicity. The formulae to be given here are simple; they fit naturally into the procedure of Russell's paper II; they are believed to be so accurate that in many cases no subsequent adjustment will be necessary of the elements that they yield; and they agree as well as they should,<sup>‡</sup> to the first degree in  $e$ , with Russell's equations that they are intended to replace.

Consider the eclipse of the brighter star by the fainter. At the instant of conjunction,  $v = 90^\circ - \omega$ , and  $r = (1 - e^2)/(1 + g)$  where  $g = e \sin \omega$ . At this instant also, the rate at which  $v$  is increasing is  $\dot{v} = n(1 + g)^2/(1 - e^2)^{3/2}$  where  $n$  is the mean motion. Let us ignore the variations of  $r$  and  $\dot{v}$  during eclipse. Then if we denote the true anomaly measured from conjunction by  $\beta = v + \omega - 90^\circ$ , and the mean anomaly measured from mid-eclipse by  $\theta'$ , we shall have with considerable accuracy that  $\beta = \theta'(1 + g)^2/(1 - e^2)^{3/2}$ . Now  $e$  is in general small unless the stars are widely separated, and then  $\beta$  remains small during eclipse. Thus  $\sin^2 \beta$  is approximately equal to  $\sin^2 \theta'(1 + g)^4/(1 - e^2)^3$ , and (23') may be written in the approximate form

$$\frac{(1 - e^2)^3}{(1 + g)^4} \cos^2 i + \sin^2 i \sin^2 \theta' = r_1^2 \frac{1 - e^2}{(1 + g)^2} \{1 + kp(k, \alpha)\}^2,$$

valid for the eclipse of the brighter star. Since  $i$  must be fairly close to  $90^\circ$ , this equation is nearly equivalent to

$$\cos^2 i' + \sin^2 i' \sin^2 \theta' = r_1'^2 \{1 + kp(k, \alpha)\}^2, \quad (3)$$

where  $(1 + g)^2 \cot i' = (1 - e^2)^{1/2} \cot i$ , and  $(1 + g)r_1' = r_1(1 - e^2)^{1/2}$ . If equation (3) is compared with equations (1) and (2) it is seen to be the same as the exact equation that would hold in a circular orbit of unit radius, having the same period and the same  $k$  as the real orbit, but having a radius  $r_1'$  for the larger star and an inclination  $i'$ . In the same way, if  $\theta''$  is the mean anomaly measured from the instant of mid-eclipse of the fainter star by the brighter, it can be shown that the light curve during that eclipse must be nearly the same as in a circular orbit having the same period and  $k$  but with an inclination  $i''$ , and a radius  $r_1''$  for the larger star, given by

$$(1 - g)^2 \cot i'' = (1 - e^2)^{1/2} \cot i, \text{ and } (1 - g)r_1'' = r_1(1 - e^2)^{1/2}.$$

Preliminary elements may therefore be obtained by following the procedure outlined by Russell,<sup>2</sup> but with the present equations (30') replacing his equations (30),

Primary minimum	Secondary minimum	
$r_1' = r_1 \frac{(1 - e^2)^{1/2}}{1 + g},$	$r_1'' = r_1 \frac{(1 - e^2)^{1/2}}{1 - g},$	$\left. \vphantom{\begin{matrix} r_1' \\ r_1'' \end{matrix}} \right\} \quad (30')$
$\cot i' = \frac{(1 - e^2)^{1/2}}{(1 + g)^2} \cot i,$	$\cot i'' = \frac{(1 - e^2)^{1/2}}{(1 - g)^2} \cot i.$	



Russell's equation (31) may be used as it stands for obtaining the first, rough, approximation to  $e \cos \omega$ ; but it should then be replaced by

$$\frac{h(1 - e^2)^{1/2}}{1 - g^2} + \tan^{-1} \frac{h}{(1 - e^2)^{1/2}} = \frac{\pi}{P} (t_2 - t_1 - \frac{1}{2}P), \quad (31')$$

where  $h = e \cos \omega$ . Equation (31') is exact<sup>7</sup> for central eclipses, and becomes very accurate\*\* in all cases provided that its left-hand member is increased by the often negligible correction

$$\frac{1}{2}h(1 - e^2)^{1/2} \cot^2 i \left\{ \frac{1}{(1 + g)^3 + (1 + g)(g + g^2 + 3h^2) \cot^2 i} + \frac{1}{(1 - g)^3 + (1 - g)(-g + g^2 + 3h^2) \cot^2 i} \right\},$$

where the quantity in curly brackets is nearly equal to two. Russell's equations (32) should be replaced by

$$r_1'' = r_1' \frac{1 + g}{1 - g}; \quad \cot i'' = \left( \frac{1 + g}{1 - g} \right)^2 \cot i'. \quad (32')$$

His equations (33), (34) and (35) are unaltered, and one has still, of course, that

$$\frac{\delta_1}{\delta_2} = \frac{1 - g}{1 + g}. \quad (4)$$

As has been mentioned, the above formulae have been obtained by adopting constant values of  $\psi$  and of  $r$ , for each eclipse, equal to their instantaneous values at the moments of conjunction. If the eclipses are wide, it should be somewhat more accurate to use, instead, values averaged over the durations of the eclipses. The equations that result from the averaged values are the same as the equations given above, except that  $g$ , in equations (30') and (32') only, should be replaced by  $g\eta$ . With sufficient approximation,  $3\eta = 2 + \zeta$ , where  $\zeta$  is the cosine of the average of  $\theta'$  and  $\theta''$  at the beginning or the end of eclipse. However, when  $e$  is large the eclipses will in general be narrow,  $\eta$  will be effectively equal to unity, and the equations as they have been given, without  $\eta$ , should be sufficiently accurate to yield good preliminary values of the true orbital elements.

As a practical test of the accuracy of the equations of this note in a particular case, a set of true elements was adopted:  $r_1 = 0.118$ ,  $r_2 = 0.098$ ,

$\cot i = 0.0201$ ,  $e = 0.506$ ,  $\omega = 330^\circ$ . From these a light curve was computed by the exact equation (23'), and from the light curve "circular" elements were obtained:  $r_1' = 0.1364$ ,  $\cot i' = 0.02312$ ,  $r_1'' = 0.0823$ ,  $\cot i'' = 0.00830$ ,  $k = 0.8305$ . The formulae (30) of Russell, when applied to  $r_1'$  and  $r_1''$ , yielded for  $r_1$  the values 0.109 and 0.110, respectively, of which the average, 0.110, is in error by nearly eight per cent because of the large value of  $e$ . The same formulae, applied to  $\cot i'$  and  $\cot i''$ , yielded for  $\cot i$  the values 0.0154 and 0.0167, respectively, the average, 0.0160, being in error by over twenty per cent. The equations (30') of the present note, when applied to  $r_1'$  and  $r_1''$ , yielded for  $r_1$  the values 0.118 and 0.120, the average, 0.119, being only one per cent in error; and when applied to  $\cot i'$  and  $\cot i''$  they yielded for  $\cot i$  the values 0.0201 and 0.0203, the average, 0.0202, being in error by only one-half of one per cent, or twenty seconds of arc.

*Summary.*—Simple and closely approximate formulae are given, valid for large values of the orbital eccentricity  $e$ , for deriving preliminary values of the true orbital elements of an eclipsing binary star from the "circular" elements of its eclipses. The formulae are equivalent to Russell's (*Ap. J.*, 36, 54 (1912)), designed for small values of  $e$ , when  $e$  is small; they are more accurate than Russell's when  $e$  is large.

\* For the unit of  $\alpha$ , which depends upon the law of darkening and upon which of the two stars is in front, the reader is referred to papers 1 through 4.

† For one such star, see reference 5, in which it is stated that for H. V. 7498,  $e$  exceeds 0.4.

‡ Exactly, if  $\eta$  is employed; otherwise, very closely.

\*\* Equation (31') as it stands gives exactly the time-interval between conjunctions; the correction has been computed from  $v$  and from the  $\beta$ 's that minimize  $\delta^2$  for the two eclipses. The value of  $\beta$  for the instant of deepest eclipse is obtained from the first and second derivatives of  $\delta^2$  with respect to  $v$  at the conjunctions. The instants  $t_1$  and  $t_2$  are those of deepest eclipse.

<sup>1</sup> Russell, *Ap. J.*, 35, 315 (1912).

<sup>2</sup> Russell, *Ap. J.*, 36, 54 (1912).

<sup>3</sup> Russell and Shapley, *Ap. J.*, 36, 239 (1912).

<sup>4</sup> Russell and Shapley, *Ap. J.*, 36, 385 (1912).

<sup>5</sup> Shapley and Swope, *Bull. Harvard Coll. Obs.*, No. 909, 5 (1938).

<sup>6</sup> Sterne, *M. N.*, 99, 451 (1939).

<sup>7</sup> Sterne, *M. N.*, 99, 662 (1939).

<sup>8</sup> Sterne, *M. N.*, 99, 670 (1939).

*GALACTIC AND EXTRAGALACTIC STUDIES, IV.  
PHOTOMETRY OF TWO LARGE SOUTHERN  
CLUSTERS OF GALAXIES*

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1. The most conspicuous deviation from uniformity in the space distribution of galaxies is shown by the clustering into large populous groups such as the widespread organization in Virgo and the more compact cluster in Coma at the north galactic pole. Something less than twenty of these rich systems, each containing hundreds of galaxies, have been reported. On the other hand, more than fifty small groups are in our records at present and hundreds are within the range of existing telescopes. They are clusters involving ten to fifty members, frequently very compactly associated and usually in fields of irregular density. The most important of these less populous groups is undoubtedly our own local aggregation, with the galactic system, the Magellanic Clouds, the Andromeda Nebula, the Sculptor and Fornax dwarf galaxies, and a few others as members.

The present communication treats of two systems of the larger type, both of them newly discovered. The significance of the detailed photometric study on Harvard plates, which has recently been completed, lies in the contribution to knowledge of

(a) the luminosity curve of galaxies (frequency distribution of total absolute magnitudes), and

(b) the mean density of matter in "unexpanded" regions of metagalactic space.

2. General information concerning the two groups is given in table 1. The equatorial and galactic coördinates refer to the centers of the clusters. The areas are given in square degrees. The magnitude limits of the plates,  $m_s$ , indicate the brightness of the faintest star clearly seen near the plate centers. The plate population refers to the total number of galaxies measured in the central twenty-five square degrees of the plate; the cluster population has been deduced after appropriate correction for the superposed images of the general field.

TABLE 1

GROUP	POSITION IN 1900			GALACTIC		CLUSTER		PLATE NUMBER	$m_s$	PLATE POPULATION
	R. A.	DEC.		LONG.	LAT.	AREA	POPULATION			
A	1 <sup>h</sup> 3 <sup>m</sup> .7	-16° 10'		116°	-77°	0.88	420	A 18691	19.3	3847
B	22 21 .4	-49° 18'		312°	-56°	0.44	300	A 20255	18.6	3479

For these two systems, as for nearly all others we have studied, the greater part of the cluster population probably lies below the limits of the available plates. We must content ourselves, therefore, with a partial survey, with a census of only the brighter members. Even the brightest objects are so faint that no safe classification of the individuals is possible. We can only say that among them are objects of the usual types—spheroidal and spiral.

3. *Group A.*—The general field of nebulae on plate A 18691, where the

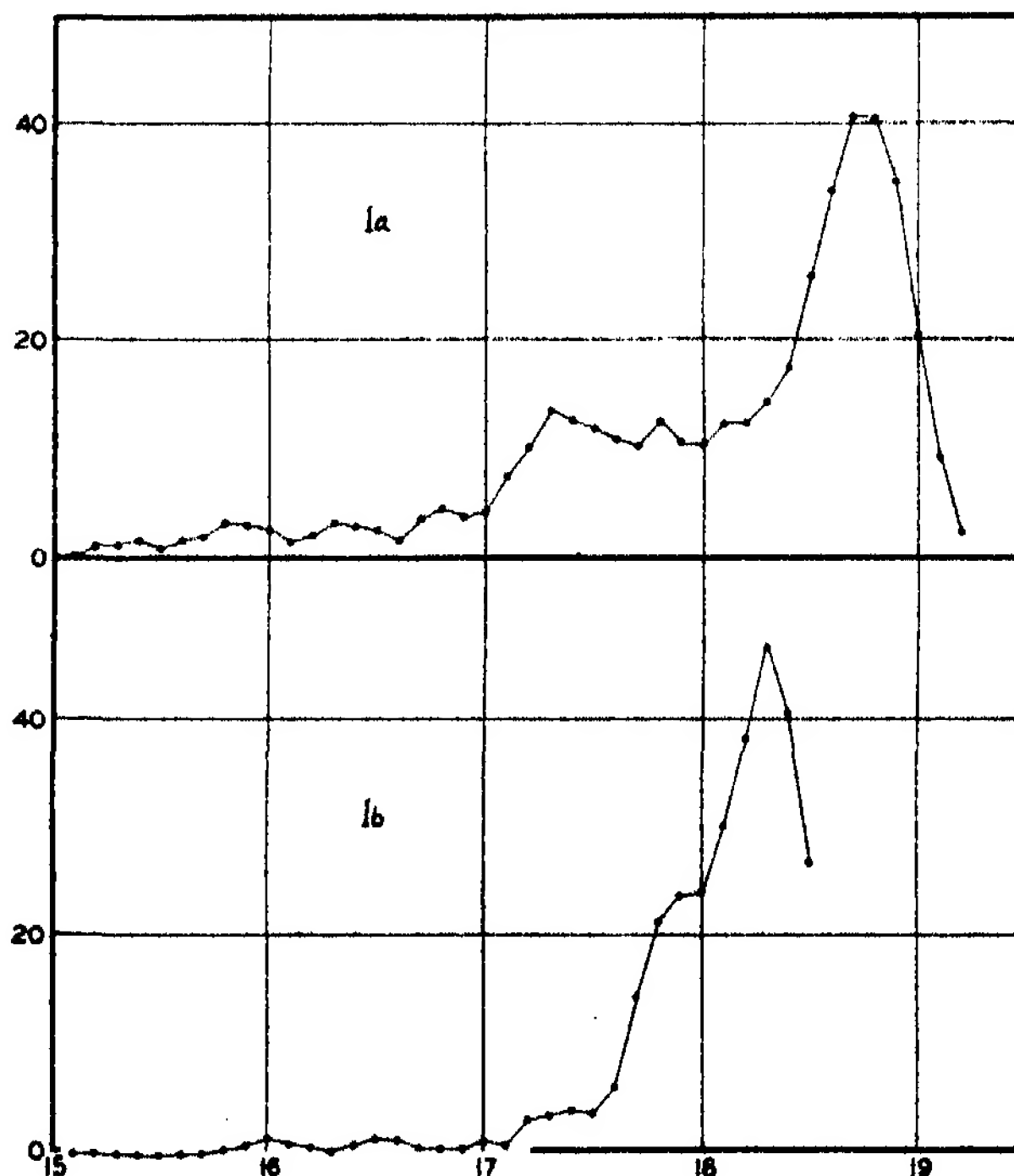


FIGURE 1

Luminosity curves for Group A (above) and Group B. Ordinates are numbers of galaxies; abscissae, apparent photographic magnitudes.

first of the two groups covers 0.88 square degrees, is very unevenly populated. In one particular square degree there are but eighty-seven objects; in an adjacent square degree, three hundred. To remove the field population from the area covered by the cluster is therefore troublesome and uncertain. The simplest and most direct procedure has been followed. First, the magnitudes in a stellar comparison sequence were established by star counts, in connection with the van Rhijn and the Seares and Joyner tables, which relate photographic magnitude to galactic coördinates and

stellar densities. With this sequence the photographic magnitudes of the nearly four thousand nebulae on the plate were twice measured to the nearest tenth of an interval. The frequency distribution of the nebular magnitudes for the eight central square degrees was next determined. To get the average field population for each tenth of a magnitude in an area equal to that covered by the cluster, the factor  $0.88/8$  was applied to the numbers for each interval. These reduced numbers for the field were then subtracted from the corresponding observed values for the cluster area and the remainders are taken as providing the true luminosity distribution for the cluster members alone.

The size of the corrections applied to the observed numbers in the cluster area is of course a function of the magnitude, and is satisfactorily represented by the relation  $N = 10^{0.6(m-16.7)}$ , where  $N$  is the number of galaxies per 0.88 square degrees to photographic magnitude  $m$ . The relation is indeed a "uniform density" formula for these particular eight square degrees.

TABLE 2  
DATA FOR LUMINOSITY CURVES, CORRECTED FOR FIELD

MAG.	GROUP A	GROUP B	MAG.	GROUP A	GROUP B	MAG.	GROUP A	GROUP B
15.0	3.8	0.6	16.4	5.5	0.7	17.9	12.8	27.1
15.0	0.0	-0.2	16.5	0.1	1.6	18.0	8.7	17.2
15.1	-0.3	-0.4	16.6	1.9	1.1	18.1	9.5	27.1
15.2	0.8	0.0	16.7	2.6	0.3	18.2	18.4	45.7
15.3	2.7	-0.3	16.8	5.9	-0.6	18.3	8.9	41.6
15.4	-0.2	-0.4	16.9	4.6	1.0	18.4	15.1	52.2
15.5	1.9	-0.4	17.0	0.9	0.3	18.5	28.6	27.8
15.6	0.8	-0.2	17.1	6.9	1.4	18.6	33.8	
15.7	1.8	-0.1	17.2	14.2	-0.1	18.7	38.7	
15.8	2.9	-0.4	17.3	8.9	7.5	18.8	49.2	
15.9	4.6	0.9	17.4	17.1	2.4	18.9	33.7	
16.0	1.4	0.7	17.5	11.8	1.7	19.0	20.8	
16.1	1.7	1.8	17.6	6.6	6.5	19.1	6.8	
16.2	1.0	-0.4	17.7	14.4	9.8	19.2	-0.1	
16.3	3.1	-0.3	17.8	10.0	26.6			

The data for the luminosity curve are given in table 2 and plotted in figure 1a, where the points are running means, each representing three-tenths of a magnitude. The curve is somewhat unusual for a cluster of galaxies. The rising branch is over 3.5 magnitudes in length, instead of the usual 2.5 magnitudes, or less. The remote possibility that a cluster of bright objects, magnitude 15 to 17.5, is superposed on a faint cluster, beginning about 17.0, has been considered. The plots in figure 2a show, however, that the center of the cluster is essentially identical for bright, intermediate, and faint objects and therefore that a single system with a wide spread in luminosity is the only reasonable hypothesis.

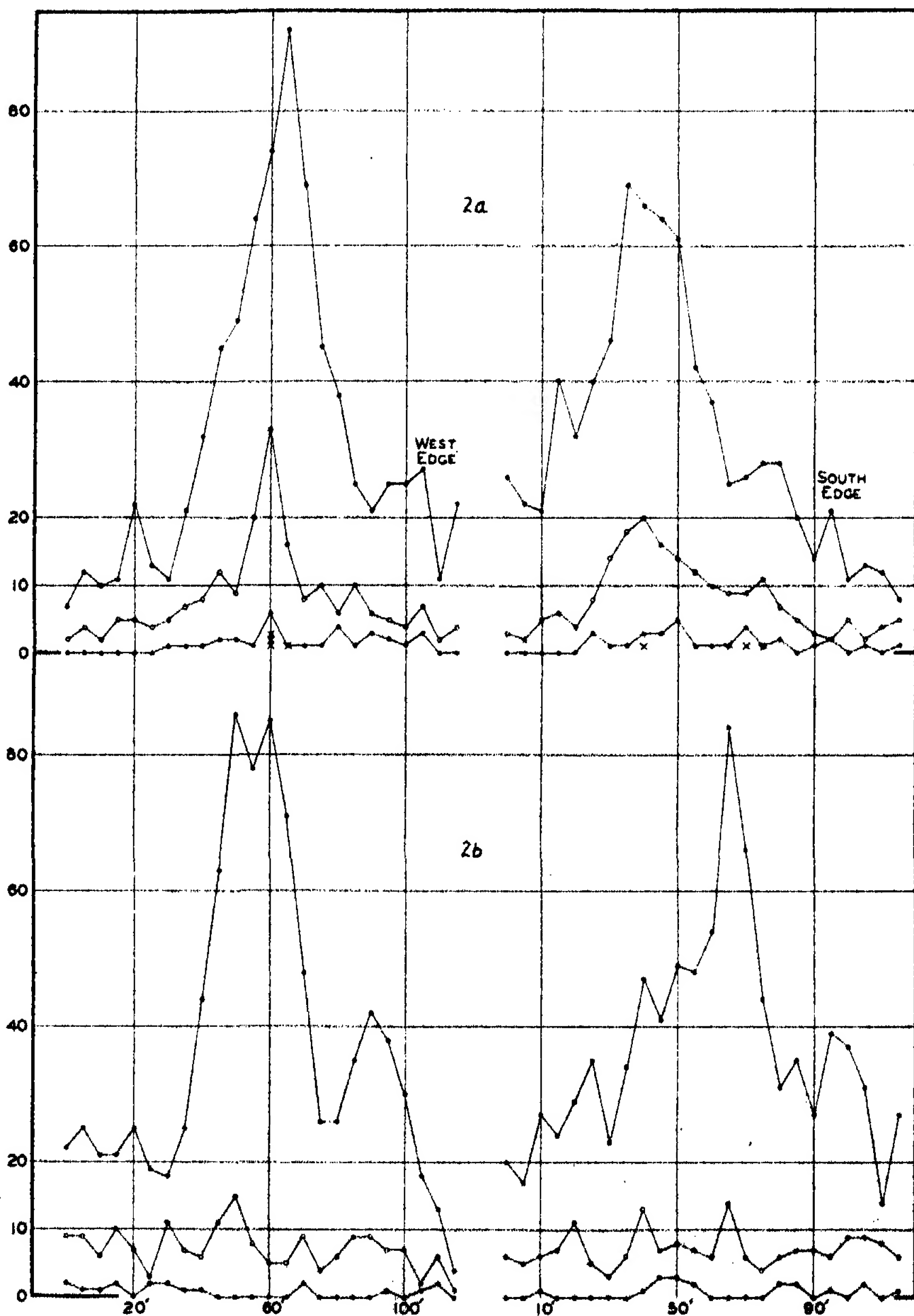


FIGURE 2

Distribution of galaxies in and around Group A (above) and Group B. Ordinates are numbers of objects in strips 5' wide and 2° long. The plots on the left represent summations for the strips running north and south, beginning at the east side of the four-square-degree areas; the figures on the right give the summations for the strips



The cluster lies in a rich region, and the distribution over the whole plate (thirty square degrees) is irregular. In fact, it is noteworthy that nearly all of the very rich clusters of galaxies are situated in regions of irregular distribution and frequently in regions of very high field density. There is in this fact an indication that a physical connection exists between heavy clustering and non-homogeneity in the field—an intimation that an earlier high space density, perhaps of the order of  $10^{-26}$  or  $10^{-27}$  g./cc., is dissolving or breaking up, and, in the environs of the cluster, approaching the more normal density of  $10^{-29}$  or  $10^{-30}$  g./cc. that appears to prevail in intergalactic space. On this hypothesis the cluster which we are now isolating and measuring represents an "unexpanded" region—a remnant or nucleus of a formerly much larger system.

Within the nearly circular area of Group *A* there is greater richness along the north-south axis. This elongation is shown by figure 2*a*, where the distribution of galaxies over four square degrees, centered on the cluster, is represented for objects fainter than photographic magnitude 17.5, and for two brighter intervals. The total numbers of galaxies in strips five minutes of arc wide and two degrees long are shown on the left for strips running north and south, and on the right for the east-west strips. The low values for the south edge of the area are probably due in part to magnitude loss because of distance from the plate center. We should expect, but do not find, a similar loss at the west edge; irregularities in the field population probably compensate for the distance effect. The north-south elongation of the denser portions of Group *A* is shown in figure 2*a*, both for objects fainter than 17.5 and those between 16.0 and 17.5, by the differences in form between north-south and east-west curves.

The positions in the cluster of the four objects with magnitude brighter than 15.5 are indicated by crosses in figure 2*a*. These bright objects (table 3) are probably the giant galaxies of the cluster, with absolute magnitudes not fainter than  $-17$ , and therefore comparable to the Andromeda Nebula in intrinsic luminosity. The linear dimensions are computed on the assumption that the distance of the group is 29 megaparsecs.

The field nebulae could not be eliminated in making figure 2, but the curves, through indicating the population of the field, provide a check on the method described above for removing field objects before making the luminosity curves in figure 1.

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FIGURE 2 (Continued)

running east and west, beginning at the north side. The plotted values have not been smoothed or corrected for the field nebulae. In each of the four sections the distribution of objects fainter than magnitude 17.5 is represented by the upper curve; magnitudes 16.0 to 17.5 inclusive, by the middle curve; and magnitudes brighter than 16.0, by the lower curve. The crosses in figure 2*a* are discussed in the text.



4. *Group B*.—The distribution of galaxies throughout the four square degrees, at the center of which is the second of the two clusters, is shown in figure 3. The positions of objects brighter than 17.5 are indicated by open circles; those of magnitude 17.5 and fainter, by dots. It is clear that there is very little clustering of bright objects, in contrast to the situation in Group *A*. The cluster, according to this diagram, would not be detectable on plates showing objects only to magnitude 17.5. Table 2 and figure 1*b* show, in fact, that when the field galaxies have been removed there remains scarcely an object brighter than magnitude 17. Beginning with magnitude 17.5, however, the luminosity curve rises steeply until the approach to the plate limit interferes with the survey. A total of three hundred cluster members has been measured. (The star-count sequences for Group *B* are based on the van Rhijn tables alone.)

If the distribution of absolute magnitudes in Group *B* is similar to that found at Mount Wilson and Harvard in other rich clusters (for example, the Coma cluster<sup>1</sup>), we should expect the maximum of the luminosity

TABLE 3  
GIANT GALAXIES IN GROUP A

DESIGNATION	CLASS	DIAMETERS , ,	PHOTOGRAPHIC MAGNITUDE	ABSOLUTE MAGNITUDE	LINEAR DIMENSIONS KPC
I 78	S	$2.1 \times 0.9$	14.4	-17.9	$18 \times 8$
I 79	E1	$0.7 \times 0.7$	14.9	-17.4	$6 \times 6$
I 82	Sb?	$0.9 \times 0.7$	14.85	-17.45	$8 \times 6$
1221	E3	$0.5 \times 0.4$	15.4	-16.9	$4 \times 3$

curve to be not brighter than photographic magnitude 18, and the total population to be well over a thousand members. Figure 2*b* shows that Group *B* is smaller in angular diameter than Group *A* and that the distribution within its nearly circular outline is not especially elongated, though decidedly irregular, so far as we can judge from the three hundred cluster members above the plate limit. To remove the effects of the field on the cluster's luminosity curve, the same procedure was used as for Group *A*, except that ten square degrees, appropriately selected to avoid differential distance-effects, were studied to determine the probable field membership in the area of 0.44 square degrees occupied by the cluster itself.

It is found that the relation  $N = 10^{0.6(m - 16.9)}$  satisfactorily represents the frequency of magnitudes for an average field of 0.44 square degree in this galactic latitude and longitude. Reducing the formula to its value for one square degree, we find a space density parameter

$$N_1 = 16.3$$

which closely agrees with the mean value for the whole of the southern galactic hemisphere in high latitudes. In other words, the average population in the vicinity of Group *B* apparently is normal, whereas for Group *A* we find  $N_1 = 16.55$ , a value indicating a density considerably below average, although not beyond the limit of common fluctuations from average density.

5. *Estimate of Distances.*—The high galactic latitude and the richness of the surrounding fields both suggest that little correction to the apparent

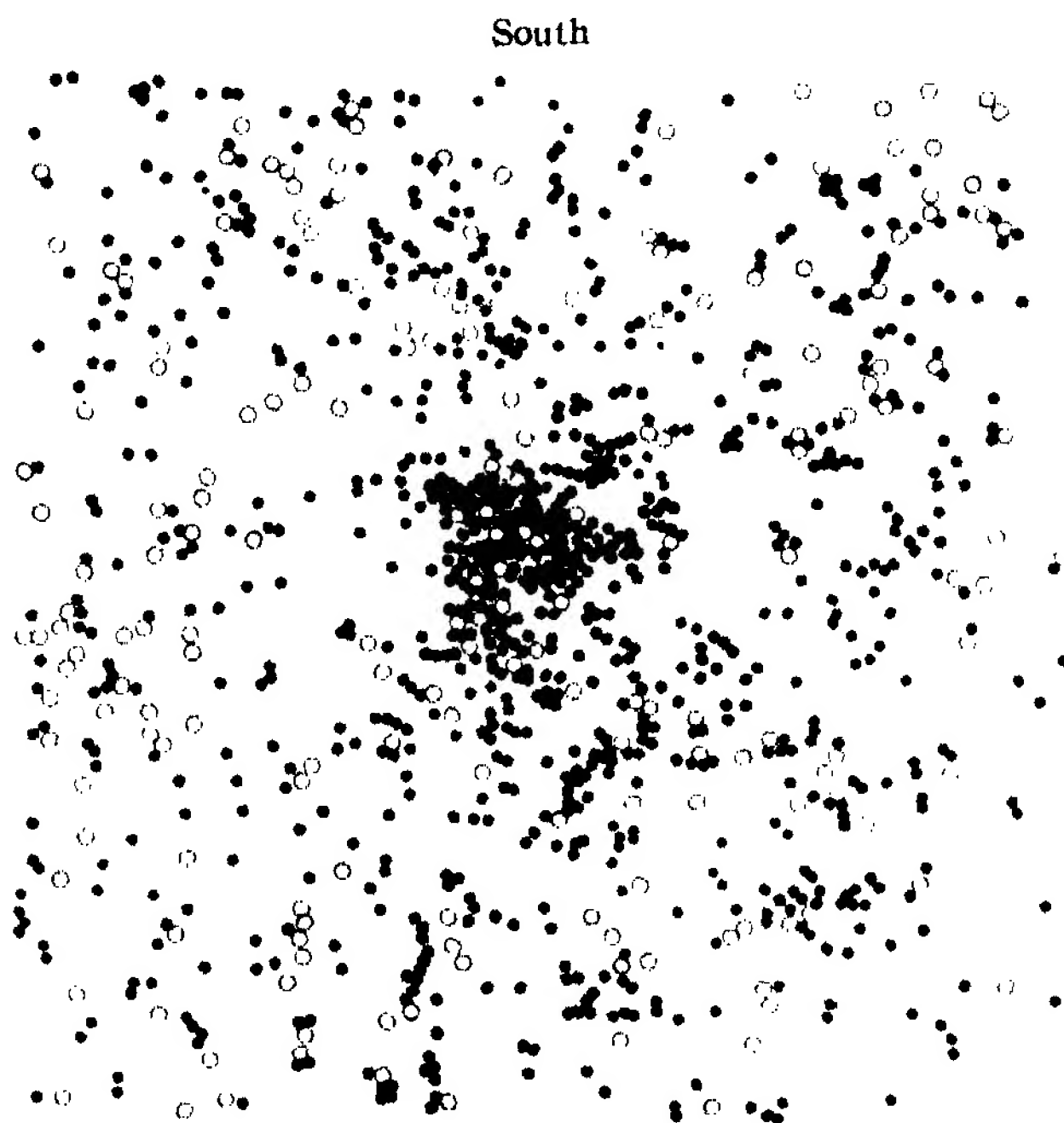


FIGURE 3

Distribution of objects in four square degrees centered on Group B. Objects fainter than magnitude 17.5 are indicated by dots; brighter objects, by open circles. Stars in this field are not plotted.

magnitudes need be made for space absorption. A small correction for the red shift is necessary since the velocity of recession for such distant clusters exceeds ten thousand kilometers a second. Following the method originally used for globular star clusters and later adapted by Hubble to the study of clusters of nebulae, we may estimate the distances of these two groups from the apparent magnitudes of the fifth brightest member or the thirtieth, as well as from the apparent magnitude at the maximum of the luminosity curve. In the following tabulation  $\Delta m_{p,\lambda}$  is the adopted

correction to the observed apparent magnitudes for space absorption and red shift;  $m_5$  is the corrected apparent magnitude of the fifth brightest cluster member;  $m - M_1$  is the resulting distance modulus when we adopt, on the basis of Harvard and Mount Wilson studies of the nearer rich clusters, the absolute magnitude  $-16.3$  for the fifth brightest object in a rich system.

GROUP	$\Delta m_{\beta, \lambda}$	$m_5$	$m - M_1$	MAXIMUM MAGNITUDE	$m - M_2$
A	0.2	15.0	31.3	18.6:	32.8
B	0.3	16.6	32.9	18.8+	32.9+

The maximum of the luminosity curve is uncertainly estimated for Group A, and is merely guessed from the shape of the ascending branch for Group B. The magnitudes corrected for absorption and red shift are in the next to the last column, and in the last is the resulting distance modulus.

Obviously the distances calculated from this material are only approximate; if they are within twenty per cent of the true values they suffice to give useful information on the luminosities and diameters of the brighter members and an indication of the space density of galaxies in these large systems. It seems appropriate to give only half-weight to the distance modulus derived from the fifth magnitude in Group A because of the unusual form of the luminosity curve. Half-weight should also be given to the value estimated from the unobserved maximum of Group B. We adopt, therefore, the mean moduli of 32.3 and 32.9 for Group A and Group B, respectively. The corresponding distances are

Group A, 29 megaparsecs  
Group B, 38 megaparsecs

With the distance as indicated for Group A, it appears certain that some of the brightest members of the cluster are comparable in total absolute magnitude with the Andromeda Nebula for which Hubble estimates the brightness at  $-17.5$ . The values of linear dimensions and absolute magnitudes are given above in table 3. If really a member of the group, one of the objects, IC 78, which appears to be a spiral on edge, with a strong nucleus, is fifty per cent larger than the Andromeda Nebula as ordinarily photographed.

In an earlier survey of magnitudes in twenty-five clusters of galaxies,<sup>2</sup> where a few groups were found to be of this very populous type, we had already indicated the presence of a few giant galaxies, some of which were approximately of the luminosity and linear dimensions of the giants here recorded.

With the distances as indicated above, the linear diameters of Group *A* and Group *B*, respectively, are 0.27 mpc. and 0.25 mpc. But it is probable that many of the objects lying further afield are also physical members of these super-systems and the true dimensions, if boundaries can be fixed, exceed a million light years.

We are indebted to Miss Frances W. Wright for making second measures on all the magnitudes and for other assistance with this research.

*Summary.*—(a) In the study of nearly eight thousand galaxies on two exceptionally good three-hour plates, made with the Bruce refractor at the Boyden Station, two rich groups have been found and measured. They bear the same relation to the ordinary small group of galaxies as globular clusters bear to galactic clusters, but the rich groups of galaxies are not highly or isometrically centralized.

(b) The distances are 29 and 38 megaparsecs for Groups *A* and *B*, respectively, and their diameters exceed two hundred and fifty kiloparsecs.

(c) The plates do not reach to the faintest objects in either system, but when appropriate correction is made for field nebulae we count approximately four hundred cluster members in Group *A* and three hundred in Group *B*, and estimate the total populations at approximately a thousand each. The corresponding space densities are probably in excess of  $10^{-27}$  g./cc.

(d) The luminosity curve deduced for Group *B* is very similar to that found for other rich systems of this sort; but in Group *A* there appears to be a considerable number of unusually bright galaxies which are almost certainly members of the system and not merely superposed field galaxies (figure 1).

(e) The giant systems in Group *A* are of both the spiral and spheroidal types. Some of them are comparable with the Andromeda Nebula in luminosity, and one of them, IC 78, a spiral on edge, appears to exceed the Andromeda Nebula both in luminosity and in linear dimensions, if it is not by chance a nearer superposed galaxy.

<sup>1</sup> *Mt. W. Contr.* No. 427, 22 (1931); No. 549, 7 (1936); *Harv. Bull.*, 896, 9 (1934).

<sup>2</sup> These PROCEEDINGS, 19, 1002 (1933).

## THE DOPPLER EFFECT IN RESONANCE LINES

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The problem of the Doppler broadening of spectral lines consists of two parts, the computation of the absorption coefficient as a function of position in the line and the determination of the distribution of the scattered light over the line. It has generally been supposed in astrophysical applications that the condition of monochromatic radiative equilibrium is present in the formation of resonance lines. That this is not the case, under conditions when Doppler broadening is present, follows from the fact that the projected velocity, and hence the Doppler shift, of an atom is not generally the same along the direction of the scattered ray as it is along the primary ray. The absorption coefficient for combined damping and Doppler broadening is well known and has been used extensively. In the present report we consider the second problem, the distribution of the scattered light over the various parts of a spectral line.

In the absence of Doppler broadening the absorption coefficient is

$$\frac{\pi e^2}{mc} N f \frac{\gamma}{\pi \gamma^2 + (\nu - \nu_0)^2}.$$

Here  $N$  is the number of atoms per gram of the gas and  $\gamma$  is the natural half breadth.

Let us now consider the scattering into a direction making an angle  $\alpha$  with the original ray. Let us choose rectangular coördinates ( $u_1, u_2, u_3$ ) such that the plane  $u_3 = 0$  contains the two rays and the  $u_1$  axis makes an angle  $\alpha/2$  with each of the two rays. An atom having velocity components ( $u_1, u_2, u_3$ ) will have a projected velocity along the primary ray

$$u_1 \cos \alpha/2 - u_2 \sin \alpha/2,$$

and along the scattered ray

$$u_1 \cos \alpha/2 + u_2 \sin \alpha/2.$$

A frequency  $\nu'$ , as referred to the moving atom, corresponds to frequencies  $\nu_1$  and  $\nu_2$  as seen along the primary and scattered rays, respectively. Their values are as follows:

$$\left. \begin{aligned} \nu_1 &= \nu' + \frac{\nu}{c} (u_1 \cos \alpha/2 - u_2 \sin \alpha/2), \\ \nu_2 &= \nu' + \frac{\nu}{c} (u_1 \cos \alpha/2 + u_2 \sin \alpha/2). \end{aligned} \right\} \quad (1)$$

The distribution of velocities for a kinetic temperature  $T$  is given by the expression

$$\left(\frac{Am_1}{2\pi kT}\right)^{3/2} e^{-\frac{Am_1}{2kT}(u_1^2 + u_2^2 + u_3^2)} du_1, du_2, du_3.$$

Here  $A$  is the atomic number and  $m_1$  is the mass of an atom of unit atomic number.

The energy absorbed per gram from an incident beam of unit intensity by the atoms in the velocity intervals  $du_1$ ,  $du_2$  and  $du_3$  is

$$\frac{\pi e^2}{mc} Nf \frac{\gamma}{\pi \gamma^2 + (\nu' - \nu_0)^2} \left(\frac{Am_1}{2\pi kT}\right)^{3/2} e^{-\frac{Am_1}{2kT}(u_1^2 + u_2^2 + u_3^2)} du_1, du_2, du_3. \quad (2)$$

This energy is scattered into the various directions according to the phase function

$$\frac{3}{16\pi} (1 + \cos^2 \alpha).$$

Let us introduce the following abbreviations:

$$b = \left(\frac{2kT}{Am_1}\right)^{1/2} \frac{\nu_0}{c},$$

$$\kappa_0 = \frac{\pi e^2}{mc} Nf \frac{1}{\sqrt{\pi b}},$$

$$a_1 = \gamma/b,$$

$$a_2 = \gamma/b \sec \alpha/2,$$

$$v_1 = \frac{\nu_1 - \nu_0}{b},$$

$$v_2 = \frac{\nu_2 - \nu_0}{b},$$

$$x = \frac{\nu_0}{cb} u_1.$$

Except for subscripts these symbols have the meanings usually ascribed to them.<sup>1</sup>

It follows from these relations and from (1) that

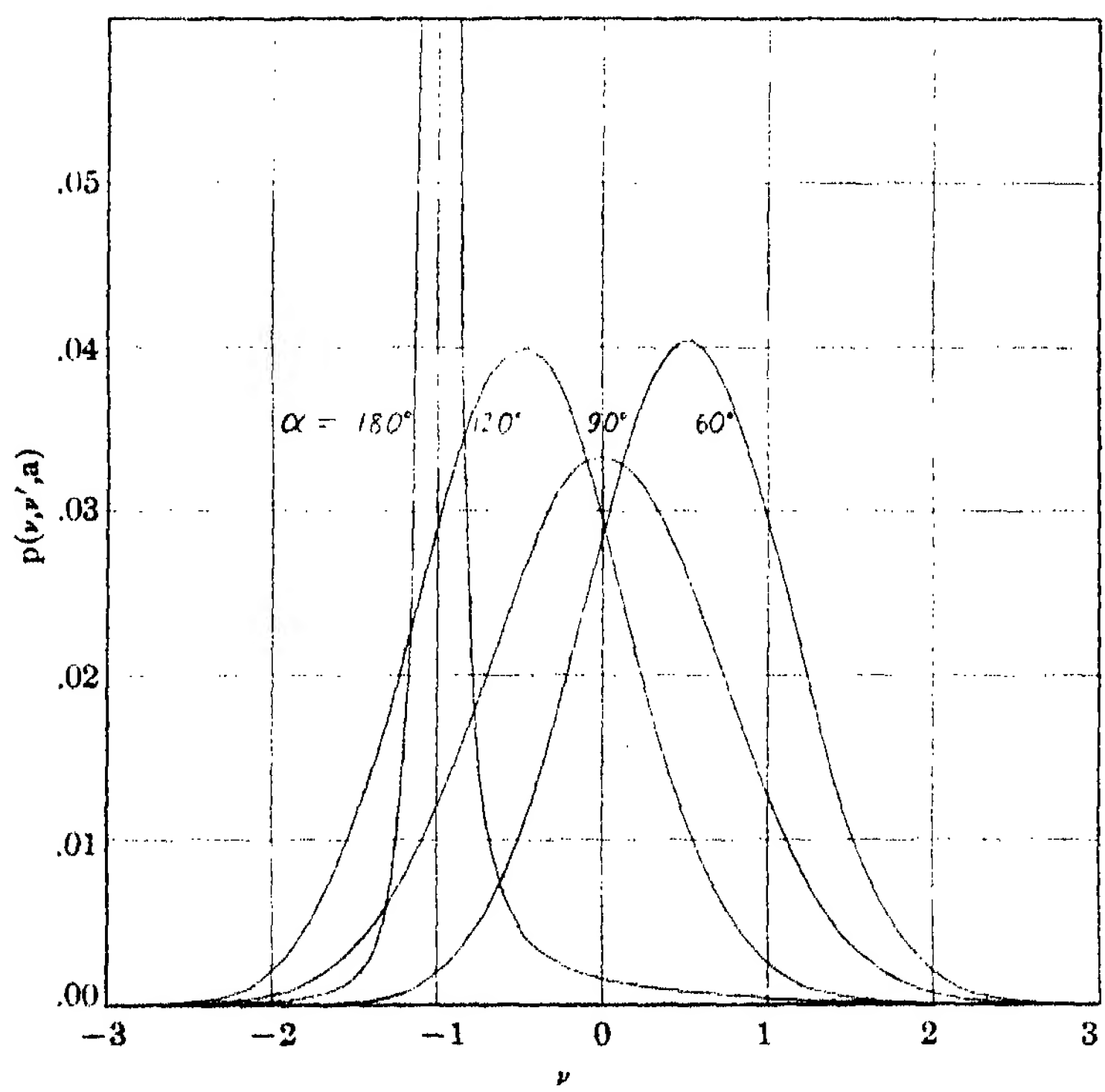
$$v_2 = v_1 + \frac{2\nu_0}{bc} u_2 \sin \alpha/2,$$

$$\frac{v_1 + v_2}{2} = \frac{\nu_1 - \nu_0}{b} + \frac{\nu_0}{bc} u_1 \cos \alpha/2.$$

TABLE 1

$\alpha \backslash \eta$	-2.5	-2.0	-1.5	-1.0	-0.5	0.0	+0.5	1.0	1.5	2.0	2.5
					$a_1 = 0.01$	$\eta_1 = 0.0$					
60°	0.0001	0.0011	0.0020	0.0104	0.0293	0.0399	0.0293	0.0104	0.0020	0.0011	0.0001
90°	0.0001	0.0007	0.0041	0.0118	0.0260	0.0335	0.0260	0.0118	0.0041	0.0007	0.0001
120°	0.0000	0.0002	0.0022	0.0109	0.0288	0.0402	0.0288	0.0109	0.0022	0.0002	0.0000
180°	0.0000	0.0000	0.0001	0.0006	0.0029	1.920	0.0029	0.0006	0.0001	0.0000	0.0000
					$a_1 = 0.01$	$\eta_1 = 0.5$					
60°	0.0002	0.0005	0.0007	0.0051	0.0191	0.0377	0.0356	0.0194	0.0052	0.0007	0.0005
90°	0.0001	0.0007	0.0035	0.0124	0.0260	0.0333	0.0261	0.0143	0.0039	0.0007	0.0001
120°	0.0001	0.0008	0.0051	0.0189	0.0367	0.0370	0.0194	0.0054	0.0009	0.0001	0.0000
180°	0.0000	0.0001	0.0004	0.0022	1.916	0.0037	0.0010	0.0004	0.0002	0.0001	0.0000
					$a_1 = 0.01$	$\eta_1 = 1.0$					
60°	0.0000	0.0000	0.0002	0.0020	0.0105	0.0286	0.0404	0.0294	0.0103	0.0021	0.0002
90°	0.0001	0.0007	0.0037	0.0129	0.0263	0.0332	0.0258	0.0121	0.0035	0.0006	0.0001
120°	0.0002	0.0021	0.0110	0.0290	0.0398	0.0293	0.0114	0.0025	0.0005	0.0001	0.0000
180°	0.0000	0.0002	0.0017	1.893	0.0047	0.0016	0.0009	0.0005	0.0003	0.0001	0.0000
					$a_1 = 0.1$	$\eta_1 = 0.0$					
60°	0.0001	0.0002	0.0022	0.0109	0.0289	0.0398	0.0289	0.0109	0.0022	0.0002	0.0001
90°	0.0001	0.0009	0.0045	0.0128	0.0254	0.0326	0.0254	0.0128	0.0045	0.0009	0.0001
120°	0.0002	0.0007	0.0034	0.0121	0.0281	0.0366	0.0281	0.0121	0.0034	0.0007	0.0002
180°	0.0003	0.0008	0.0021	0.0064	0.0274	0.2120	0.0274	0.0064	0.0021	0.0008	0.0003
					$a_1 = 0.1$	$\eta_1 = 0.5$					
60°	0.0000	0.0001	0.0007	0.0049	0.0183	0.0367	0.0370	0.0203	0.0066	0.0007	0.0001
90°	0.0001	0.0008	0.0036	0.0115	0.0244	0.0315	0.0263	0.0153	0.0050	0.0013	0.0003
120°	0.0002	0.0011	0.0056	0.0177	0.0326	0.0344	0.0210	0.0084	0.0023	0.0007	0.0002
180°	0.0003	0.0010	0.0037	0.0208	0.2060	0.0342	0.0102	0.0043	0.0020	0.0010	0.0004
					$a_1 = 0.1$	$\eta_1 = 1.0$					
60°	0.0000	0.0000	0.0002	0.0018	0.0094	0.0265	0.0392	0.0314	0.0135	0.0031	0.0005
90°	0.0002	0.0006	0.0030	0.0104	0.0222	0.0306	0.0294	0.0157	0.0066	0.0027	0.0005
120°	0.0003	0.0020	0.0093	0.0232	0.0288	0.0292	0.0158	0.0065	0.0027	0.0012	0.0005
180°	0.0009	0.0041	0.0258	0.2392	0.0547	0.0184	0.0089	0.0047	0.0025	0.0013	0.0006







The first of these equations shows that for a given initial frequency and phase angle the final frequency is uniquely determined by  $u_2$ . The total energy scattered into the frequency  $\nu_2$  is therefore given by the integration of the contributions of all velocities  $u_1$  and  $u_3$ . Equation (2) gives these contributions since the energy which is absorbed must be emitted in its entirety. Substituting all of our abbreviations and integrating over all values of  $u_1$  and  $u_3$ , we arrive at the following expression for the coefficient of scattering:

$$\frac{3}{16\pi^{1/2}} \frac{1 + \cos^2 \alpha}{\sin \alpha} \kappa_0 d\nu_2 e^{-\left(\frac{\nu_2 - \nu_1}{2}\right)^2 \cos^2 \alpha/2} \times \frac{a_2}{\pi} \int_{-\infty}^{\infty} \frac{e^{-x^2} dx}{a_2^2 + \left(\frac{\nu_1 + \nu_2}{2} \sec \alpha/2 - x\right)^2} \quad (3)$$

As an alternative representation we may consider the fraction of the energy which, after being absorbed at  $\nu_1$  appears at  $\nu_2$  in unit interval of frequency and in direction  $\alpha$  in unit solid angle. We get this fraction by dividing by the absorption coefficient  $k$  and by  $d\nu_2$ , and we have

$$p(\nu_1, \nu_2; \alpha) = \frac{1}{b} \frac{3}{16\pi^{1/2}} \frac{1 + \cos^2 \alpha}{\sin \alpha} \frac{\kappa_0}{\kappa} e^{-\left(\frac{\nu_2 - \nu_1}{2}\right)^2 \cos^2 \alpha/2} \times \frac{a_2}{\pi} \int_{-\infty}^{\infty} \frac{e^{-x^2} dx}{a_2^2 + \left(\frac{\nu_1 + \nu_2}{2} \sec \alpha/2 - x\right)^2} \quad (4)$$

In case  $\alpha = 0$ , that is, the light is scattered forward, the energy should appear at exactly the same frequency as that at which it was absorbed. This fact also follows from the preceding formulae. Allowing  $\alpha$  to approach zero we can verify that

$$p(\nu_1, \nu_2; 0) \begin{cases} \longrightarrow 0 & \text{if } \nu_1 \neq \nu_2, \\ \longrightarrow \infty & \text{if } \nu_1 = \nu_2. \end{cases}$$

In Table 1 we give a number of values of the quantity given in formula (3). In order to illustrate the effects which are involved, the values for  $a_1 = 0.01$  and  $\nu_1 = 1$  are also reproduced in the adjoining diagram. It appears from this diagram that the distribution of the scattered light over frequency has maxima which progressively approach, with increasing phase angle, a position which is exactly opposite the primary frequency. For phase angle zero the frequency is, of course, unchanged while for phase angle  $\pi$  the shift is a maximum. In the event that the primary frequency is well outside of the core of the line ( $\nu_1 \gg 1$ ) the situation is different. In this case the Doppler shifts are negligibly small compared with the distance

from the center of the line and we may expect that the scattered light will be distributed closely about the primary frequency. These considerations may be studied directly from our formulae using the known properties of the functions involved therein.

<sup>1</sup> Cf. Hjerting, *Ap. Jour.*, 88, 508 (1938).

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## THE THEORY OF THE VISUAL THRESHOLD. I. TIME AND INTENSITY

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I. In the development of the theory of photic excitation in organisms a place of especial significance has been occupied by the application of the Roscoe-Bunsen Rule. The rule states that for the production of a fixed quantity of photochemical change the product of intensity and exposure time is constant.

Three chief types of evidence have been concerned in testing the validity of this "reciprocity rule" for photic stimulation. In one, exposure times have been determined for minimal response in 50 per cent of individuals at different intensities of illumination.<sup>1</sup> In the second, the relation between intensity and exposure time for the threshold of excitation has been measured in single individuals.<sup>2</sup> Experiments of a third type have involved a modification of the Roscoe-Bunsen Rule, namely, the Talbot-Plateau Law according to which the photosensory effect of a continuous light is equalled by that of light periodically interrupted with sufficiently high frequency if the intensity is increased so that the mean flux is the same.<sup>3</sup> The physical meaning of the data in experiments of these three kinds actually differs in certain interesting ways, however.<sup>4</sup>

II. We are concerned here only with data of the second type. To the extent to which it can be shown that the Reciprocity Rule is obeyed for the visual threshold, the evidence could be taken to support the notion that the sensory effect at the threshold is due to a constant amount of photochemical disturbance.<sup>5</sup> It has thus an important connection with the common doctrine of the constancy of the effects responsible for discriminatory differences, especially since the same considerations have also been applied to differential thresholds  $\Delta I = I_2 - I_1$  as a function of exposure-time.<sup>6</sup>

The existing data relevant to this matter are numerous. They are of two main kinds. A large number of series of measurements show the product of threshold intensity ( $\Delta I_0$ ) and exposure time ( $t$ ) to pass through a minimum.<sup>7</sup> Other series show  $(\Delta I_0) \times (t)$  "constant" up to a critical time,<sup>8</sup> beyond which  $\Delta I_0$  approaches constancy. No entirely comprehensive survey of this situation seems to have been made. For consistency's sake it would seem desirable to restrict the use of photochemical considerations in the interpretation of visual data to those measurements involving time-intervals so short as may be known to give approximate constancy in the  $I \times t$  product under the conditions; but this would depend upon the assumption that  $I \times t$  is really a constant when not complicated by observational errors and when not obscured by the effects of a "critical time" for the delivery of excitation.<sup>8</sup> The explanation of the increase of the  $I \times t$  product with longer times has been made in terms of the balance of light—and dark—reactions.<sup>5</sup> This does not account for those data in which  $I \times t$  passes through a minimum.

The effort has sometimes been to explain the minimum in  $(\Delta I_0) \times (t)$  as due to technical errors in timing, or to ocular movements. These suggestions are not convincing; nor are they really necessary. The condition for a minimum in the product is obviously that the relative rates of change of  $\Delta I_0$  and of  $t$  should be numerically equal but of opposite sign—namely, at the point of  $45^\circ$  slope in the curve of  $\log \Delta I_0$  vs.  $\log t$ . If no such point is included in the range of  $\Delta I_0$  and  $t$ , which is partly determined by the area of the test-patch used and the wave-length composition of the light, or if the time-intervals are too widely spaced, no minimum will be apparent. A theoretical interpretation is required which will embrace the full range of the  $\Delta I_0, t$  data and which will permit the physical evaluation of the rôle of other variables, for example, of retinal area.

The situation is, of course, qualitatively identical in certain essential particulars with that presented by the phenomena of electrical excitation in nerve, as regards the approach to constant quantity of electricity for excitation as a limit for short times, and in the exhibition of an energy minimum.<sup>9</sup> It is also seen in the deviation from the Reciprocity Rule for blackening of a photographic plate.<sup>10</sup> Into these parallels we need not now go, beyond pointing out that they can probably all be brought under the same kind of explanation.<sup>4</sup>

III. Reasons of several kinds have been advanced<sup>11</sup> for taking  $1/I$ , where  $I$  is the exciting intensity, as the measure of excitability. For threshold excitability we have  $1/\Delta I_0$ . The threshold intensity acts upon an assemblage of units of which the momentary capacities for excitation form some kind of a frequency-distribution of  $dk$ , where  $k$  is a velocity constant. The dependence of  $1/I$  upon the temperature in responses to visual flicker at constant flash frequency shows<sup>11</sup> that  $1/I$  behaves as

if governed by the activation of a catalytically controlled intrinsic reaction velocity, to which  $1/I$  is directly proportional. The units constituting a homogeneous set exhibit this velocity in statistical distribution, but with the same *kind* of control in each. Under these conditions a velocity constant has the meaning of a reciprocal time. The excitability at the point of threshold response, with continuous exposure, is measured by  $1/\Delta I_0$ . In the critical excitation of flicker, flashes of intensity  $I_c$  are presented repetitively, a large number of times, for single durations  $= t = 1/F$ , where  $F$  is the flash frequency. The critical  $I_c$  has therefore the meaning of a  $\Delta I_0$ ; the conditions of its recognition as a distinct flash are different from those imposed when a single, non-repeated flash is used,<sup>12</sup> but the nature of  $I_c$  as essentially the equivalent of a  $\Delta I_0$  is sufficiently

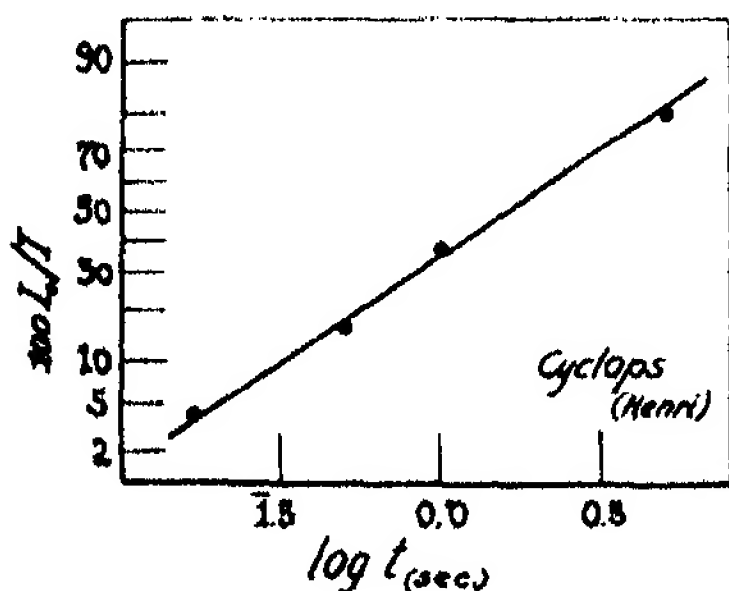


FIGURE 1

Reciprocal threshold intensity for phototropic effect (ultra-violet light) as a percentage of the reciprocal of a minimum  $I_0$ , as a function of log exposure time, for *Cyclops*.<sup>10</sup> The ordinate scale is that of a probability integral.

clear.<sup>13</sup> Excitation is the result of the production of impulses by units exhibiting a frequency distribution of instantaneous thresholds, and thus of  $d(1/I)$ ; the number of impulses to be obtained over a finite time from a unit briefly remaining in any given frequency class will be a declining function of intensity above its threshold intensity (accommodation), and consequently the frequency distribution of these impulses will be in terms of  $-I.d(1/I)$ , and thus of  $d \log I$ .<sup>14</sup> But  $1/I$  exhibits<sup>11</sup> the properties of  $k$ , proportional to  $1/t$  if the temperature is constant, and hence we must expect that  $d \log I \equiv cd \log t$ . The peculiar quantity signified by the

logarithm of a *time* is thus understandable in terms of the proportionality of *time* and the reciprocal of a *velocity constant*. Consequently we expect to find  $1/\Delta I_0$  measured by the summation of the impulses provided in the integral of the frequency distribution of  $d \log t$ . The form of this function is suspected to be a normal probability integral.<sup>4</sup> The reasons, briefly, are: (1) the individual elements are nerve impulses; (2) the incidence of an impulse is ruled by probability considerations in a given excitable unit;<sup>15</sup> these elements are quantal, and their effects add to produce a total effect; moreover, the relations of effect to temperature show that, in nerve,<sup>16</sup> and as for  $\log I$  in visual responses,<sup>11</sup>  $\sigma_{\log t}$  can be independent of mean  $\log t$  and is not determined by it. Consequently all the assumptions basic to the derivation of a Gaussian curve<sup>17</sup> appear to be specifically justified in this particular case.

IV. The kinds of experimental quantitative modification to which the 3 parameters of this particular function can be put<sup>18</sup> provide the deciding

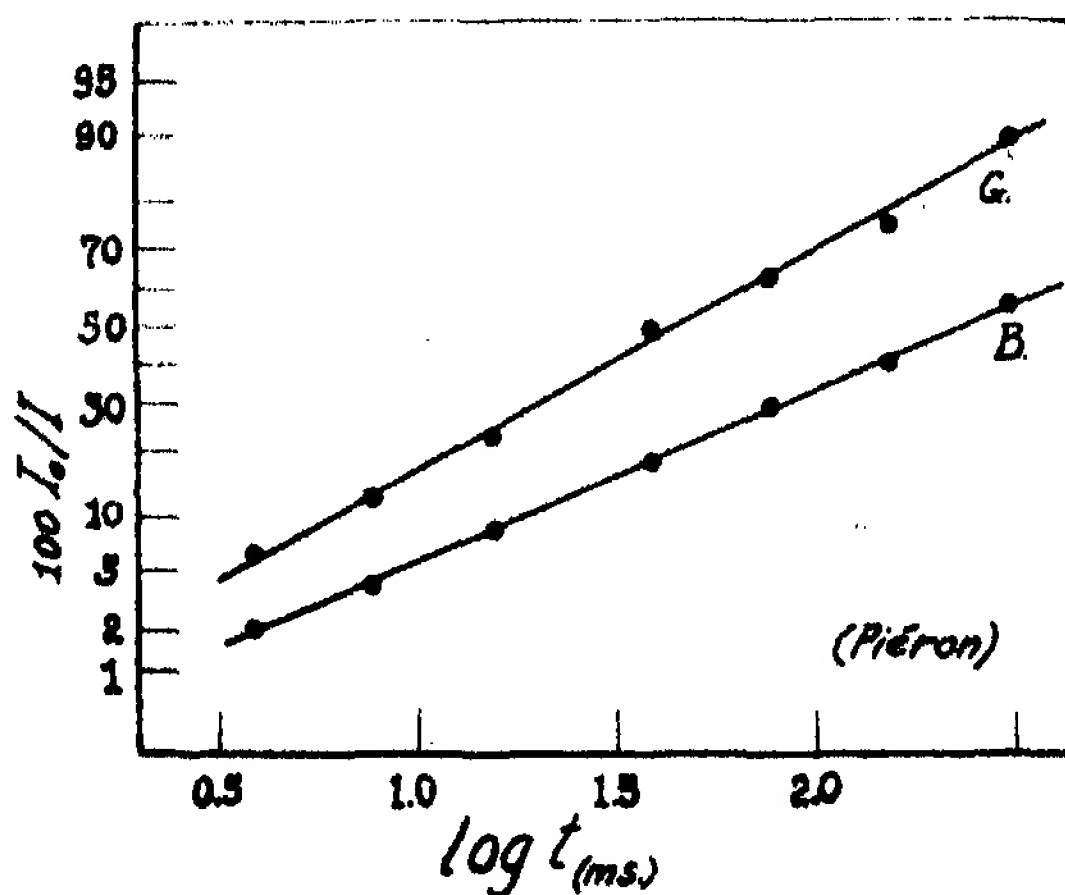


FIGURE 2

Representative data for green (G) and for blue (B) light, from Piéron,<sup>20</sup> showing that the relations between  $1/\Delta I_0$  and  $\log t$  are satisfactorily rectified in a probability grid.

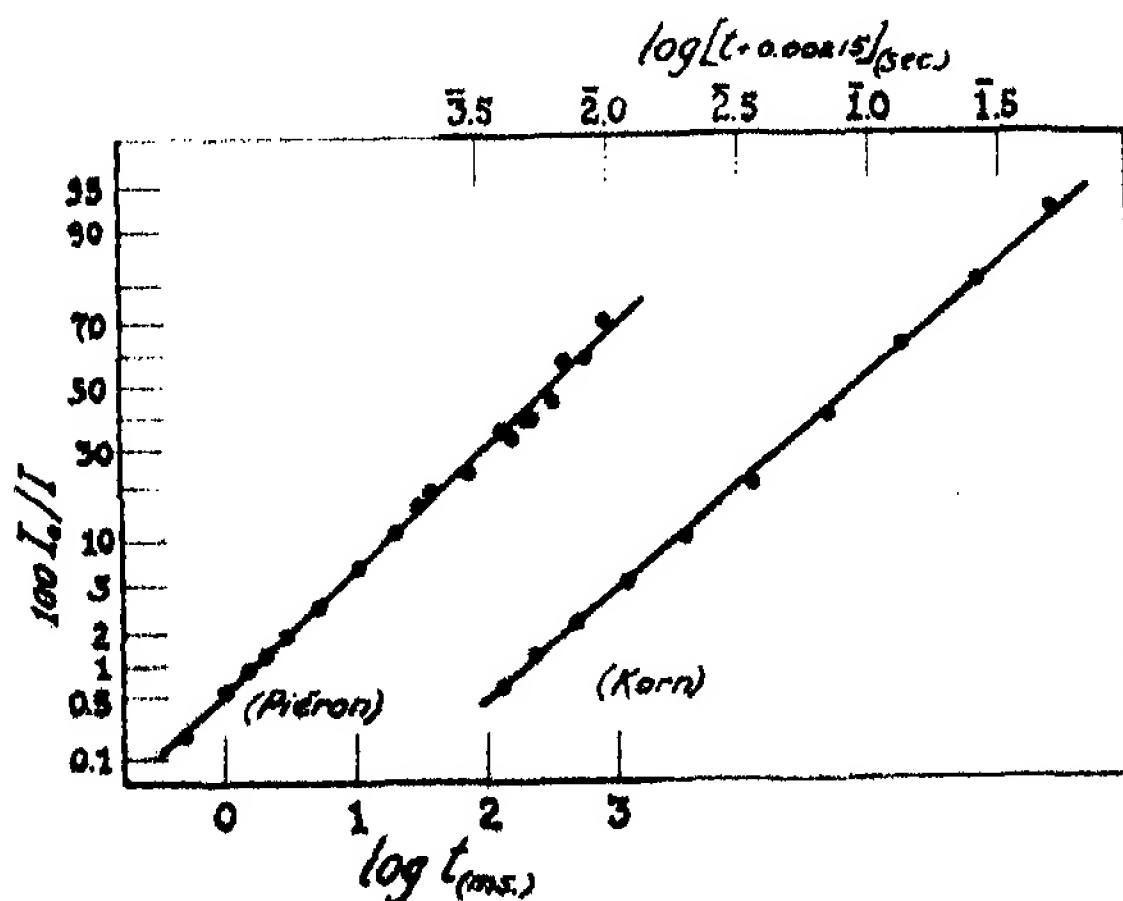


FIGURE 3

Data from Piéron<sup>20</sup> and from Karn,<sup>21</sup> on a probability grid (see text).

test of its propriety. Its application to a number of really homologous instances superficially of quite different sorts is considered at some length



in another place,<sup>4</sup> and has been referred to briefly in an earlier note.<sup>16</sup> The purpose here is to illustrate the application to the complete range of some representative sets of measurements, including several for which the  $I \times t$  product goes through a minimum. When the complete range can be accounted for, there is no necessity to invoke a "critical" intermediate time. The illustrations are taken from data of considerable range which there is reason to regard as adequately homogeneous.

In figure 1 there are given data from Henri and Henri<sup>19</sup> for the phototropic threshold of *Cyclops* (ultra-violet light). Figures 2 and 3 give several series of data from Piéron<sup>7</sup> and one from Karn.<sup>20</sup> Numerous other series

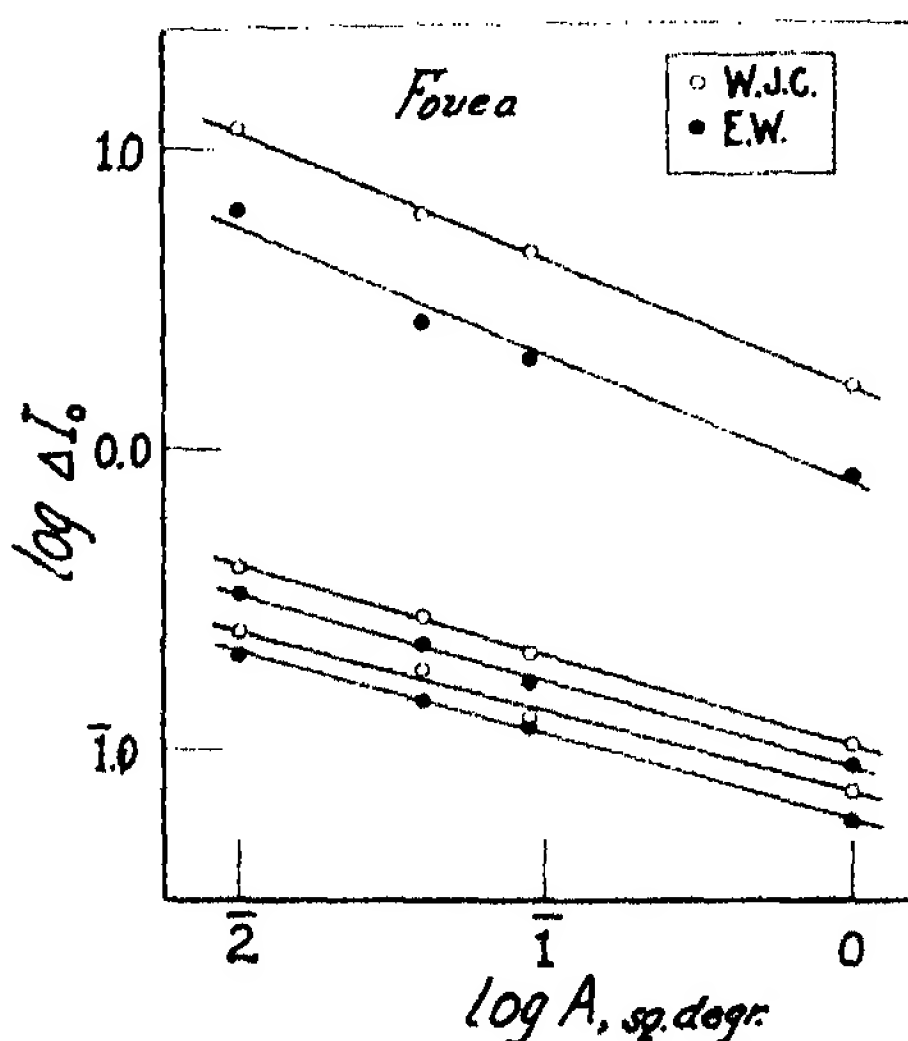


FIGURE 4

Measurements of threshold intensity as a function of area, within the fovea, for two different observers, at three different exposure times (cf., figure 5).

$\sigma'_{\log t}$  should remain the same so long as the area concerned is really homogeneous, although the maximum value of  $1/\Delta I_0$  must increase and the abscissa of the inflection point must decrease. If, however, the area is sufficiently enlarged so that factors making for *interaction* become implicated, and consequently there is increased variability of performance in the individual elements, then  $\sigma'_{\log t}$  must *increase*. Systematic application of this test has been made in a number of regions of the retina. Results for the fovea are given in figures 4 and 5. They illustrate a significant procedure for the measurement of the extent of retinal area, in different locations, which is critical for the initiation of interaction effects.

of available data have been treated in the same way. The data from Karn illustrate one consequence of the fact that the binocular threshold, used in this series, is fundamentally lower than the mean monocular threshold;<sup>21</sup> a given intensity therefore behaves with binocular presentation as if it were acting for a longer time than when presented monocularly—hence the time measured behaves as if in reality somewhat longer.

V. It is instructive to consider the way in which the  $I-t$  function is modified when the retinal area illuminated is changed. It is clear that if in a particular region of the retina the area of the test patch is enlarged,

The correlation of such critical areas with known or identifiable structural conditions, not necessarily in the retina, presents a number of significant possibilities.

In figure 4 it is apparent that  $\Delta I_0$  is a declining power function of the area of the test patch,<sup>22</sup> and that the exponent is a decreasing function of exposure time. The same measurements are given in figure 5 upon a probability grid. They show that when the centrally fixated area approaches 1 sq. degree, interaction effects of the sort already specified make their appearance. The systematic examination of this matter will be considered elsewhere.

VI. *Summary.*—The complete range of data relating the visual intensity threshold to the exposure time can be quantitatively accounted

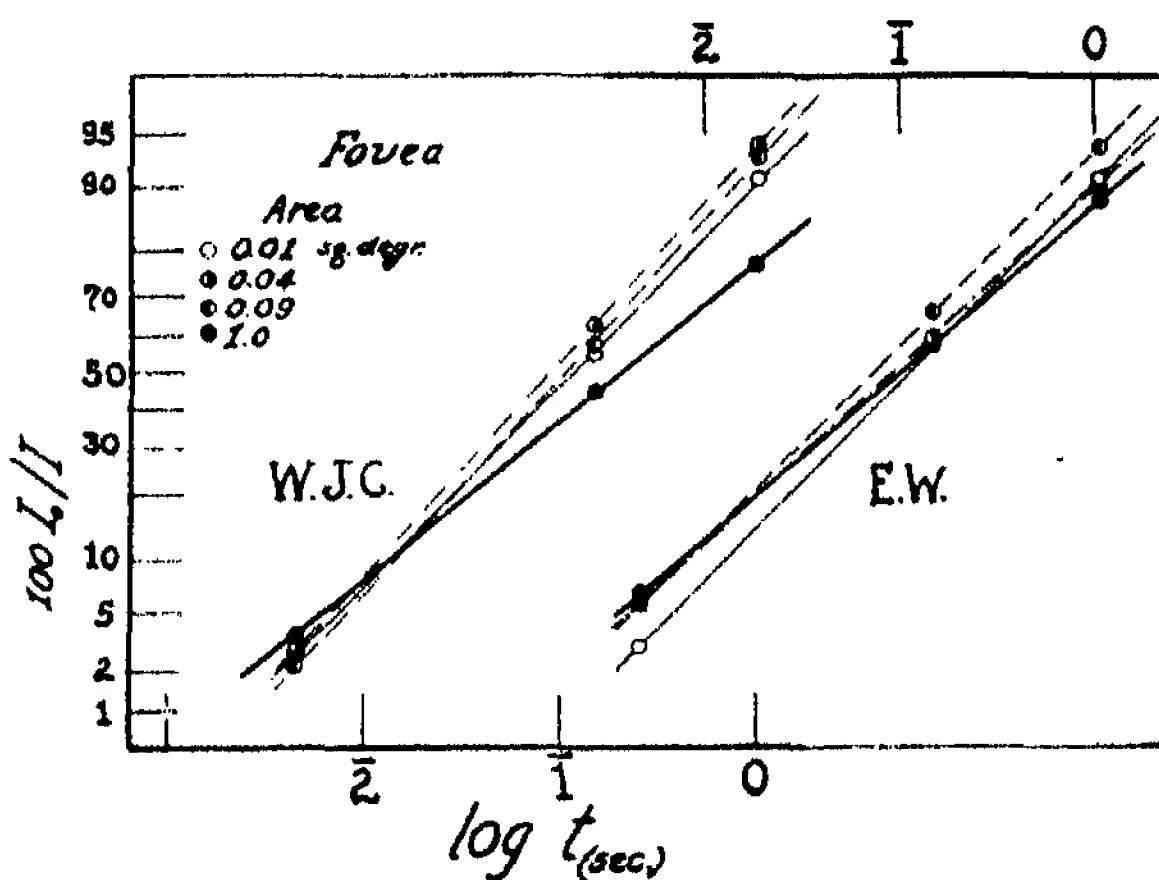


FIGURE 5

The data of figure 4 on a probability grid; see text.

for, with homogeneous measurements, in terms of the theoretical deduction that the reciprocal of the threshold intensity,  $1/\Delta I_0$ , gives a probability integral in terms of  $\log t$ . It is unnecessary to suppose that the reciprocity rule has any reasonable application to such measurements. It cannot be deduced from such data either that the sensory effect for threshold occurrence is in any sense a constant, or has anything to do with a constant amount of photochemical change.

Certain properties of the parameters of the probability integral are commented upon, particularly in relation to the area of the retinal image. It is indicated that there arise some testable deductions concerning "interaction."

<sup>1</sup> Blaauw, A. H., *Rec. trav. bot. néerl.*, 5, 209 (1909); Loeb, J., *Forced Movements, Tropisms, and Animal Conduct*, Lippincott, Philadelphia and London, 209 pp. (1918).

<sup>2</sup> Hecht, S., *Jour. Gen. Physiol.*, 2, 229, 337 (1919-1920); Piéron, H., *Compt. rend. acad. sci.*, 170, 525, 1203 (1920).

<sup>3</sup> Cf. Loeb, J., *Forced Movements, Tropisms, and Animal Conduct*, Lippincott, Philadelphia and London, 209 pp. (1918); Hecht, S., and Wolf, E., *Jour. Gen. Physiol.*, **15**, 369 (1931-1932); Gilmer, T. E., *Jour. Opt. Soc. Amer.*, **27**, 386 (1937).

<sup>4</sup> Crozier, W. J., "Theory of the Visual Threshold" (to be published elsewhere).

<sup>5</sup> Hecht, S., Chap. 14 in: *A Handbook of General Experimental Psychology*, pp. 704-828, Worcester, Clark Univ. Press (1934).

<sup>6</sup> Graham, C. H., and Kemp, E. H., *Jour. Gen. Physiol.*, **21**, 635 (1937-1938).

<sup>7</sup> Piéron, H., *Ann. Physiol.*, **15**, 116 (1939).

<sup>8</sup> Graham, C. H., and Margaria, R., *Amer. Jour. Physiol.*, **113**, 299 (1935).

<sup>9</sup> Cf. Hill, A. V., *Proc. Roy. Soc. (Lond.)*, **B 119**, 305, 440 (1936).

<sup>10</sup> Cf. Krohn, E., *Jahrb. Phot.*, **28**, 6, etc. (1914).

<sup>11</sup> Cf. *Jour. Gen. Physiol.*, **22**, 311, 487, 795 (1938-1939); **23**, 143; in press (1939-1940); and *Proc. Nat. Acad. Sci.*, **25**, 78 (1939).

<sup>12</sup> Cf. *Jour. Gen. Physiol.*, **19**, 503 (1935-1936); **20**, 393 (1936-1937).

<sup>13</sup> Crozier, W. J., and Holway, A. H., *Proc. Nat. Acad. Sci.*, **23**, 23 (1937); **24**, 130 (1938); *J. Gen. Physiol.*, **23**, 101 (1939-1940).

<sup>14</sup> The frequent utility of a logarithmic transformation for "normalizing" a distribution has long been known and employed in various connections; but this has been done merely by way of the *assumption* that thresholds, for example, are normally distributed in terms of  $\log I$ .

<sup>15</sup> Pécher, Ch., *Compt. rend. soc. biol.*, **122**, 87 (1936); **124**, 839 (1937).

<sup>16</sup> *Proc. Nat. Acad. Sci.*, **23**, 71 (1937).

<sup>17</sup> Cf. Whittaker, E. T., and Robinson, G., *The Calculus of Observations*, 2nd ed. Blackie and Son, Ltd., London (1926).

<sup>18</sup> Crozier, W. J., and Wolf, E., *Jour. Gen. Physiol.*, **23** (1939-1940) (in press); *Proc. Nat. Acad. Sci.*, **26**, 60-67 (1940).

<sup>19</sup> Henri, V. et Mme., *Compt. rend. soc. biol.*, **72**, 992 (1912).

<sup>20</sup> Karn, H. W., *Jour. Gen. Psychol.*, **14**, 360 (1936).

<sup>21</sup> Crozier, W. J., and Holway, A. H., *Jour. Gen. Physiol.*, **22**, 341 (1938-1939); **23**, 101 (1939-1940).

<sup>22</sup> Crozier, W. J., Holway, A. H., and Wolf, E., to be published in detail elsewhere.

## TEMPERATURE AND THE CRITICAL INTENSITY FOR RESPONSE TO VISUAL FLICKER. IV. ON THE INVARIANCE OF CRITICAL THERMAL INCREMENTS, AND THE THEORY OF THE RESPONSE CONTOUR

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1. Experimental evidence discussed in earlier publications has been held to demonstrate certain invariant properties of the flicker-response contour. This contour is defined by the measured relation of flash-frequency  $F$  to flash-intensity  $I$  critical for response of various animals to visual flicker. The contour, for a specified animal, at a fixed temperature,

and with a given proportion of light-time in the flash-cycle of a given form, is a recoverable function in repeated tests.<sup>1</sup> It is properly described as a band with margins  $F \pm \sigma_F$  and  $I \pm \sigma_I$ , enclosing the region of functional dependence within which homogeneous determinations of the interrelationship of  $F$  and  $I$  will be found to occur with a given probability.<sup>2</sup>

$F$  is fixed at various levels (or  $I$ ), and critical values of  $I$  (or reciprocally of  $F$ ) are experimentally determined for single individuals<sup>3</sup> or for members of a demonstrably homogeneous population,<sup>4</sup> by means of a uniform and widely applicable technique.<sup>5</sup> In cases uncomplicated by the visual duplexity evidenced with most vertebrates,<sup>6</sup> and in an arthropod<sup>7</sup> with opic surfaces sufficiently flat so that mechanical conditions of photic reception do not introduce distortion of the performance contour,<sup>8</sup> the curve relating mean  $I$  to  $F$  (or mean  $F$  to  $I$ ) is efficiently and precisely described by a probability integral in  $\log I$ .<sup>6,7</sup> This formulation provides an efficient<sup>9</sup> and justifiable<sup>10</sup> procedure for the analytical dissection of the duplex response contour of typical vertebrates into two parts,<sup>11</sup> each with a physically distinguishable basis.<sup>10</sup> It also provides a rationalization of the asymmetrical curve obtained with most arthropods.<sup>8</sup> The use of a logarithmic probability integral is also supported by the fact<sup>12</sup> that the three parameters of this function are empirically modified by different factors in ways concordant with the general ideas leading to its application.<sup>12</sup>

2. In the derivation of the logarithmic probability integral for the  $F$ -log  $I_m$  curve, and for dynamically similar instances of very diverse character involving the performance of biological systems,<sup>13</sup> use has been made of the idea in the case of photic excitation that *excitability* is properly measured by the reciprocal of the critically exciting intensity ( $1/I$ ).<sup>13</sup> It has been shown that when the temperature of the organism is altered the  $F$ -log  $I$  curve is not changed in shape, or in the maximum value of  $F$  to which (with fixed light-time cycle-fraction) the animal will respond.<sup>14</sup> The curves merely move toward higher intensities as the temperature is made lower.<sup>14</sup> The magnitude of the shift, as a function of temperature, is characteristic of the kind of animal.<sup>15</sup> The direction and the size of the shift require the interpretation that  $1/I$  for a fixed level of effect ( $F$ ) behaves as if controlled by catalytically governed reaction velocities common to all the neutral elements in the population concerned in the determination of the response.<sup>13,16</sup>

A deduction of this kind may be in a significant way justified if it can be shown that the measure of temperature-dependence is not quantitatively modified when certain other variables of the situation are experimentally made to have different values. From this standpoint the properties of the  $F$ -log  $I$  contour are of peculiar interest, in 2 distinct respects. These have to do with (a) the theory of the logarithmic probability integral for the relation between  $F$  and  $I$ , and (b) the conception of

the meaning of quantitatively specific temperature-dependence in biological systems in general.

3. The theory of the basis for the descriptive utility and predictive value of the logarithmic probability integral derives from the general proposition that  $d(1/I)$ , where  $I$  is the critical intensity for response to visual flicker, is frequency-distributed in a certain manner, but that  $-kI \cdot d(1/I)$  gives a frequency distribution of the sensory effects produced.<sup>13</sup> If  $1/I$  is governed by the velocities of intrinsic chemical events determining the excitability of the elements concerned in the manifestation of the index response, then  $1/I_m$  for a homogeneous population of such elements, with a fixed magnitude of responsive discrimination, should be rectilinearly proportional to the exponential of  $-\mu/RT$ , where  $R$  is the gas constant,  $T$  = Kelvin temperature and  $\mu$  = apparent energy of activation per mole of controlling substance (i.e., the *temperature characteristic*<sup>16</sup>). That  $\mu$  is independent of the shape of the  $F$ -log  $I$  contour, although dependent on the nature of the animal tested<sup>17</sup> (and thus on its specifically relevant chemical organization<sup>18</sup>), has been amply demonstrated. These facts are clearly confirmatory of the general proposition. It is also required to show, however, that if the form and position of the  $F$ -log  $I$  contour can be modified by some other means than change of temperature,  $\mu$  should still be the same provided the primary excitabilities have not been modified (but only the frequencies with which the excitable units contribute to the determination of the end result).

This test is easily made by utilizing the changes produced in the  $F$ -log  $I$  curve when the light-time fraction in the flash-cycle is altered. These changes are non-specific as to direction and form, although specific in amount.<sup>12</sup> When the percentage light-time ( $t_L$ ) is reduced, at constant temperature, the curve rises to a higher maximum  $F$ , its abscissa of inflection is lowered, both being in rectilinear proportion to the percentage dark-time; the value of  $\sigma_{\log I}$  with  $F_{\max.} = 100$  per cent is not a function of  $t_L/t_D$ .<sup>12</sup> Consequently we must predict that at any flash-frequency the value of  $\mu$  for  $1/I$  must be the same with different proportions of light time to dark time, since the latter influences  $k$  but not  $1/I$  in the expression  $-kI \cdot d(1/I)$ .

This test has for several reasons been made with turtles of the form *Pseudemys ssp.* The response contour for these animals with purely cone retinas is simple, but the Arrhenius plot of  $1/I_m$  exhibits a break at  $29.5^\circ$ .<sup>19</sup> Between  $12^\circ$  and  $30^\circ$   $\mu$  is quite high (26,500 cal.). The results are summarized in figure 1 for  $t_L = 10$  per cent, 50 per cent and 90 per cent of the flash cycle, at  $F = 25/\text{sec}$ . It is apparent that although the  $F$ -log  $I$  contour is altered in the ways already described,  $\mu = \text{ca. } 12,400$  above  $29.5^\circ$  and  $\mu = 26,500$  from  $12^\circ$  to  $29.5^\circ$  describes the temperature dependence of  $1/I$  without reference to the magnitude of  $t_L/t_D$ .



The reasons for the manner in which change of  $t_L/t_D$  alters the  $F$ -log  $I$  curve have already been discussed.<sup>20</sup> It is necessary to suppose that

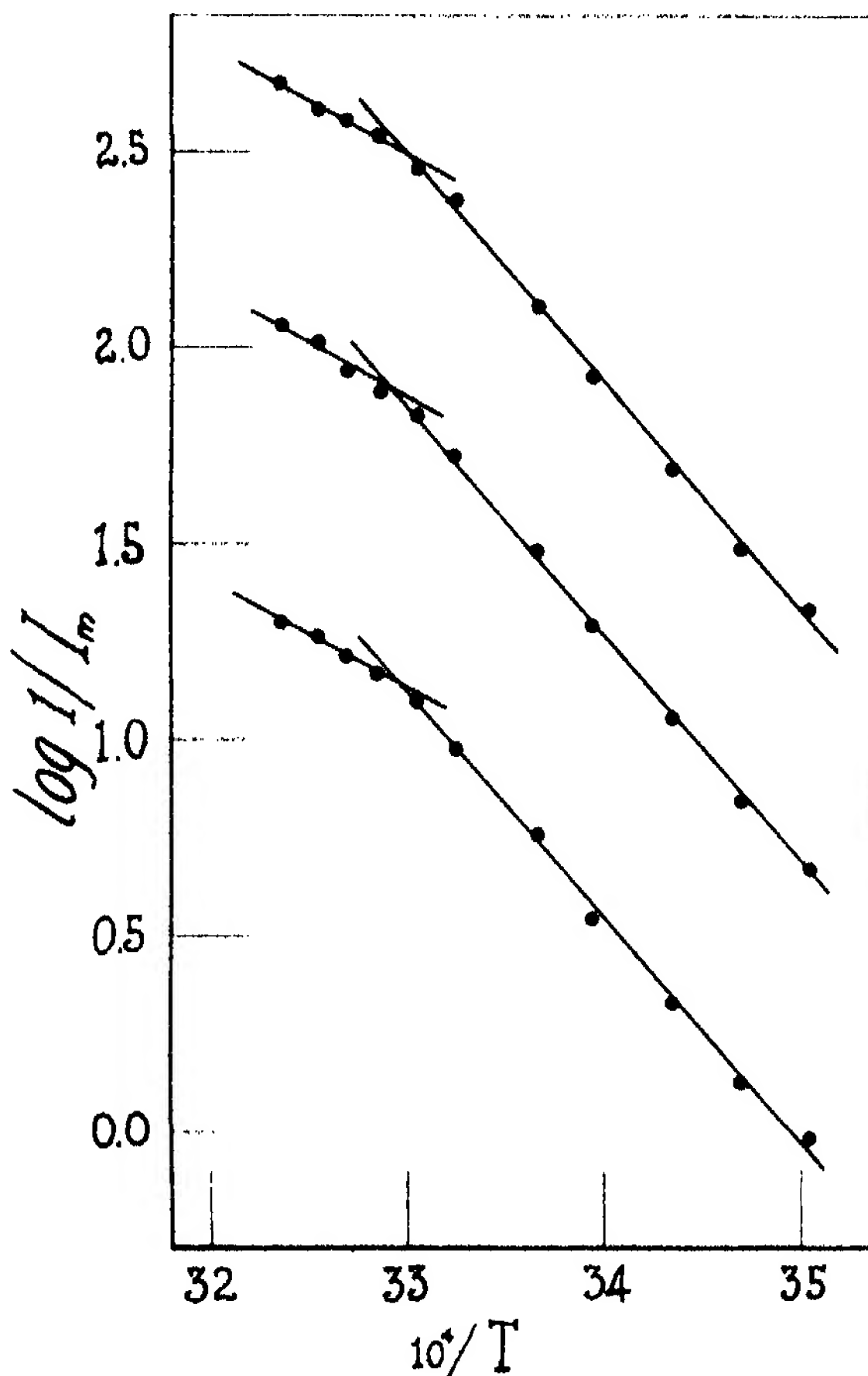


FIGURE 1

Values of  $\log 1/I_m$  critical for response to visual flicker in the dark-adapted turtle *Pseudemys*, as a function of temperature; 1 flash-frequency was used ( $F = 25$ ) at each of three values of the light-time fraction, 10 per cent, 50 per cent and 90 per cent. Each plotted entry is the mean of 30 determinations. The critical increments characteristic for  $1/I_m$  are independent of the alteration in the  $F$ -log  $I$  curves induced by changing the light-time fraction, as shown by the fact that the slopes of the lines on this graph are invariant. These slopes give  $\mu = 12,400$  above  $30^\circ$  and  $26,500$  below.

with prolongation of dark-time each flash has a greater chance to excite because more elements are likely to be non-refractory; hence the total

number of elements potentially usable over a finite time is enlarged, and thus  $f_{\max.}$ ; but the excitability of each element remains the same, and  $\sigma'_{\log I}$  is not affected, nor  $\mu$ .

4. The conception that for many vital processes the form and the magnitude of the dependence on temperature indicate control through activation of a particular catalyst<sup>21</sup> implies that the value of the temperature characteristic should be independent of the constant-temperature magnitude of the velocity concerned. In the present instances  $1/I_m$  measures the velocity of processes determining excitability. By choices of  $F$  its value can be made to vary over a range of 1:250,000 without change of the slopes of the  $\mu$  plots.<sup>22</sup> In the data of figure 1  $1/I_m$  is changed by a factor of 23 without altering  $\mu$ . These properties demonstrate the important invariant nature of the temperature characteristics when the chemical organization of the system has not been modified.

5. *Summary.*—When the light-time fraction in the flash-cycle is altered it is found that the maximum to which the flicker-response contour rises is directly proportional to the percentage dark-time; the abscissa of inflection of the  $F$ - $\log I_m$  curve decreases in the same proportion, but  $\sigma'_{\log I}$  is unchanged. For a given flash-frequency, however, the temperature characteristic of  $1/I_m$  with the turtle *Pseudemys* is quantitatively independent of the percentage dark-time, and of the flash-frequency. This variance of  $\mu$  has significance for the theory of photic excitability, and for the derivation of the probability integral relating  $F$  to  $\log I$ .

<sup>1</sup> Cf. Crozier, W. J., and Wolf, E., *Jour. Gen. Physiol.*, **22**, 463, 487, 795 (1938-1939), etc.; and a more complete account of the present experiments, to be published elsewhere.

<sup>2</sup> Crozier, W. J., *Jour. Gen. Physiol.*, **19**, 503 (1935-1936); Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Ibid.*, **20**, 211, 363 (1936-1937); **21**, 203 (1937-1938); **22**, 311 (1938-1939).

<sup>3</sup> *Jour. Gen. Physiol.*, **21**, 203 (1937-1938); **22**, 311 (1938-1939).

<sup>4</sup> Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Jour. Gen. Physiol.*, **20**, 211, 363 (1936-1937); **21**, 17 (1937-1938); Crozier, W. J., and Wolf, E., *Ibid.*, **23**, 1 (1939-1940).

<sup>5</sup> Wolf, E., and Zerrahn-Wolf, G., *Jour. Gen. Physiol.*, **19**, 495 (1934-1935); Crozier, W. J., Wolf, G., and Zerrahn-Wolf, G., *Ibid.*, **20**, 211 (1935-1936).

<sup>6</sup> Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Jour. Gen. Physiol.*, **22**, 311 (1938-1939); Crozier, W. J., and Wolf, E., *Ibid.*, **22**, 555 (1938-1939).

<sup>7</sup> Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Jour. Gen. Physiol.*, **22**, 451 (1938-1939).

<sup>8</sup> Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Jour. Gen. Physiol.*, **22**, 451 (1938-1939); Crozier, W. J., and Wolf, E., *Ibid.*, **23**, 1 (1939-1940); *Biol. Bull.*, **77**, 126 (1939).

<sup>9</sup> Crozier, W. J., *Proc. Nat. Acad. Sci.*, **23**, 71 (1937); Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Jour. Gen. Physiol.*, **21**, 17, 203, 313 (1936-1937), etc.

<sup>10</sup> Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Proc. Nat. Acad. Sci.*, **23**, 516, 542 (1937); *Jour. Gen. Physiol.*, **21**, 17 (1937-1938); Crozier, W. J., and Wolf, E., *Ibid.*, **22**, 463 (1938-1939); *Proc. Nat. Acad. Sci.*, **25**, 176 (1939).

<sup>11</sup> Cf.,<sup>9</sup> and Crozier, W. J., and Wolf, E., *Jour. Gen. Physiol.*, **23**, 229 (Nov. 1939).



<sup>12</sup> Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Jour. Gen. Physiol.*, **20**, 393, 411 (1936-1937); **21**, 223, 313, 463 (1937-1938).

<sup>13</sup> Cf. Crozier, W. J., *Proc. Nat. Acad. Sci.*, **26**, 54-60 (1940).

<sup>14</sup> Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Proc. Nat. Acad. Sci.*, **24**, 216 (1938); Crozier, W. J., *Ibid.*, **25**, 78 (1939); Crozier, W. J., and Wolf, E., *Ibid.*, **25**, 171, 176 (1939); *Jour. Gen. Physiol.*, **22**, 487, 795 (1938-1939); **23** (Nov. 1939) (in press).

<sup>15</sup> Cf. *Jour. Gen. Physiol.*, **22**, 487 (1938-1939), etc.

<sup>16</sup> Crozier, W. J., *Jour. Gen. Physiol.*, **7**, 123 (1924-1925); *Proc. Nat. Acad. Sci.*, **10**, 461 (1924).

<sup>17</sup> Crozier, W. J., and Wolf, E., *Proc. Nat. Acad. Sci.*, **25**, 171 (1939); *Jour. Gen. Physiol.*, **23** (Nov. 1939) (in press).

<sup>18</sup> Data on the frequency of breathing movements in two teleost genera and their hybrids, to be discussed elsewhere.

<sup>19</sup> Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Proc. Nat. Acad. Sci.*, **24**, 125, 538 (1938); *Jour. Gen. Physiol.*, **22**, 311 (1938-1939).

<sup>20</sup> Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Jour. Gen. Physiol.*, **20**, 363 (1936-1937); **21**, 313 (1937-1938).

<sup>21</sup> Cf. Crozier, W. J., *Jour. Gen. Physiol.*, **7**, 189 (1924-1925); **9**, 531 (1925-1926); Hadidian, Z., and Hoagland, H., *Ibid.*, **23**, 81 (1939-1940).

<sup>22</sup> Crozier, W. J., and Wolf, E., *Jour. Gen. Physiol.*, **22**, 487, 795 (1939-1940).

<sup>23</sup> We are under obligation to Mrs. E. Wolf for assistance in the experiments. The more complete account of the work, to be given elsewhere, will include consideration of the variation of  $1/I$  within and between individuals.

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## ON SPINAL SHOCK

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High reflex excitability and high extensor tone have been observed in the hind legs of cats after the recovery of an isolated caudal part of the spinal cord from certain periods of asphyxia (van Harreveld and Marmont<sup>1</sup>). Since the increased reflex excitability remained in a number of animals for the rest of their lives (up to three weeks), it was concluded that this was caused by a release of the spinal reflexes from a spinal inhibitory mechanism, which in the normal animal depresses them. It was assumed that this inhibitory mechanism was more sensitive to asphyxia than the excitatory structures and that by certain periods of asphyxia the inhibitory mechanism can be eliminated more or less exclusively.

The reflex excitability of the isolated normal spinal cord thus seems to depend on an equilibrium between the functions of excitatory and inhibitory structures. The more active the inhibitory structures, the more the reflex excitability will be depressed, eventually to total areflexia.

Spinal shock, a state of diminished reflex excitability or even of areflexia, which is pronounced only in the primates, might be due to such a dominance of an inhibitory mechanism in the spinal cord. The present experiments were undertaken to see whether, as would be predicted from the above, it is possible to curtail spinal shock by asphyxiating the cord. The monkey, *Pithecus rhesus*, in which Hinsey and Markee<sup>2</sup> recently investigated the spontaneous recovery from shock, was used.

In nine monkeys the spinal cord was severed by a ligature round the dura at Th. 9-Th. 10, thus isolating the caudal part of the spinal cord and dural cavity. Directly after finishing the operation, 10 to 20 minutes after the ligation of the cord, the pressure in the isolated part of the dural cavity was raised above the blood pressure by forcing into it through a canula, physiological solution under a pressure of 24–25 cm. mercury. In this way the blood was prevented from entering the vessels of the cord, for periods between 15 and 30 minutes. After this the reflexes of the hind limbs were examined frequently.

After this procedure a large number of reflexes have been observed often of high, sometimes even of extreme sensitivity. In the following discussion of the results all times are reckoned from the ligation of the cord. In all the experiments brisk tendon reflexes (knee- and ankle-jerks) have developed within a few hours. In many experiments tone was observed in the extensor muscles, sometimes of considerable height, developing within an hour; it has even been possible to record an appreciable stretch reflex from the gastrocnemius group after 4 hours. Skin reflexes appeared in most animals. Movements of the toes elicited by stimulation of the sole (plantar response) were observed after 2 to 3 hours. The flexor reflex in the hind leg and reflex contractions of the tail appeared in many animals after periods ranging from a few hours to 24 hours. The tendon reflexes and tone returned in most animals only temporarily, disappearing after having been manifest for periods between a few hours and 48 hours. In two animals, however, the tendon reflexes remained for life (6 days in both cases). The skin reflexes once developed remained in most cases for the rest of the period of observation (3 to 10 days). In some animals they were of an extreme sensitivity during that entire period.

Hinsey and Markee in a recent paper reported the period of absence of a number of reflexes after transection of the cord in 7 monkeys (*Pithecus rhesus*). They found that the knee-jerk returned after 1 to 20 days (average 7 days). In some cases no ankle-jerk was ever observed, in others this reflex returned after 11 to 62 days. The plantar response was seen after 1 to 20 days (average 6 days) and the flexor reflex after 4 to 102 days (average 27 days). After spontaneous recovery from shock the spinal reflexes are at the beginning very small and it is usually weeks until they reach an appreciable height (Fulton and Sherrington<sup>3</sup>).

Since, as we have shown, quite high reflex activity is often observed beginning a few hours after combined transection and asphyxiation of the cord, there can hardly be any doubt that asphyxia can curtail spinal shock. This return of reflex activity of the isolated and asphyxiated caudal part of the cord as described above is a strong support for the conception of spinal shock as a dominance of an inhibiting structure in the spinal cord itself.

It must be assumed that the reflex activity of the normal animal depends on a continuous depressing influence of the higher centers on the inhibitory mechanism in the spinal cord. The spinal shock following transection of the cord must be considered as a release of that spinal inhibitory mechanism from this depressing higher control, resulting in a diminution and eventual abolition of reflex excitability.

Acknowledgment is gratefully made for assistance from the Neurological Fund of the California Institute of Technology.

<sup>1</sup> van Harreveld, A., and G. Marmont, *Jour. Neurophysiol.*, 2, 101-111 (1939).

<sup>2</sup> Hinsey, J. C., and J. E. Markee, *Jour. Comp. Neurol.*, 69, 471-485 (1939).

<sup>3</sup> Fulton, J. F., and C. S. Sherrington, *Jour. Physiol.*, 75, 17-22 (1932).

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## PHYSIOLOGY OF DEVELOPMENT OF THE FEATHER. IV. THE DIURNAL CURVE OF GROWTH IN BROWN LEGHORN FOWL

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### I. INTRODUCTION

Evidences of daily periodicity of functions in fowl are abundant; in this paper we add to them a series of measurements demonstrating that the rate of growth of regenerating feathers is not constant during a 24-hour period, but exhibits considerable fluctuations characterized especially by a very low rate during part of the night. The physiological causes and the morphological consequences of this phenomenon will be discussed after presentation of the data.

Rates of growth during the entire period of regeneration of feathers have been determined several times (cf. Juhn and Gustavson, '30; Juhn, Faulkner and Gustavson, '31; and Lillie, '40). In all these cases the measurements were not less than 24 hours apart; but an analysis of the diurnal distribution of 24-hour increments has not hitherto been attempted.

Owing to limitations of the methods of measuring, it was decided that intervals of less than six hours would be impractical. Accordingly, a first

series of measurements of the growth in six-hour intervals in a continuous 24-hour period was made from the base of twelve o'clock. This series was then repeated at a later date. These measurements showed a very marked decline in growth-rate in the period from midnight to six A. M. The measurements did not, however, necessarily determine very closely the time of lowest or highest rates. Accordingly, two other sets of six-hour increments were prepared using nine o'clock as base. Comparison of the results from the twelve o'clock base and the nine o'clock base enables a closer estimation of the time of minimum growth in a 24-hour period.

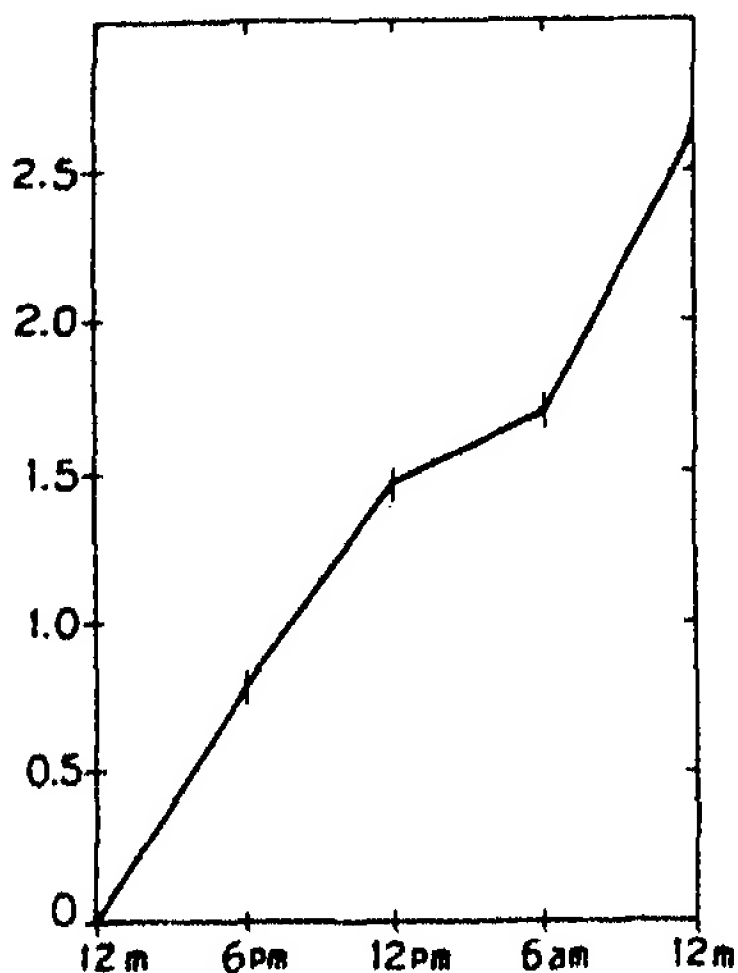


FIGURE 1

Breast feathers. From data of table 1. The grand average of six-hour increments of the three birds plotted. Based on 12:00 o'clock. Ordinates = millimeters of growth; abscissae = time of day.

each period; the grand average of these increments is plotted in figure 1.

These measurements were then repeated on the same feathers on the 28th day of regeneration (table 2), and similarly averaged. The first and second sets of measurements did not differ significantly.

Determinations of six-hour increments in the growth of saddle feathers (posterior region) were made on the same birds in an exactly similar manner on the 25th day of regeneration (April 18-19, 1939) and again on the 28th day of regeneration (tables 3 and 4). As in the case of the breast, ten feathers identified by number, with individual records for each feather, were used in each bird. Both sets of determinations agreed very closely.

## II. THE DATA

1. *Six-Hour Increments Based on Twelve O'Clock.*—Ten actively regenerating feathers of the same age in a small area of the breast of each of three Brown Leghorn capons were isolated by removing adjacent feathers; each feather was identified by number and individual records were kept. On the 25th day of regeneration (March 18-19, 1939) measurements were made of the length of each feather at six-hour intervals beginning at noon and terminating at noon of the next day. The increment in length of each feather during each six-hour period was determined from these measurements (table 1). The increments of the individual feathers, 12:00 noon to 6:00 P. M., 6:00 P. M. to 12:00 midnight, 12:00 midnight to 6:00 A. M., 6:00 A. M. to 12:00 noon, were then averaged for

TABLE 1<sup>1</sup>

TWELVE O'CLOCK BASIS. MARCH 18-19, 1939. BREAST FEATHERS BROWN LECHORN  
CAPONS. TWENTY-FIFTH DAY OF REGENERATION

		1 12 NOON	2 6 P. M.	INC.	3 12 P. M.	INC.	4 6 A. M.	INC.	5 12 NOON	INC.
Capon	1	37.9	38.2	0.3	39.7	1.5	39.7	0.0	40.0	0.3
148	2	37.8	37.9	0.1	38.9	1.0	39.2	0.3	39.6	0.4
	3	36.5	37.1	0.6	37.1	0.0	37.8	0.7	39.2	1.4
	4	34.7	36.0	1.3	36.1	0.1	36.1	0.0	36.9	0.8
	5	35.1	35.6	0.5	36.5	0.9	37.6	1.1	37.7	0.1
	6	35.0	35.6	0.6	36.5	0.9	36.8	0.3	37.6	0.8
	7	36.7	37.2	0.5	38.6	1.4	39.2	0.6	39.8	0.6
	8	36.6	36.5	-0.1*	37.0	0.5	37.5	0.5	38.8	1.3
	9	35.6	36.1	0.5	36.9	0.8	37.3	0.4	38.4	1.1
	10	35.5	36.1	0.6	37.0	0.9	37.1	0.1	38.4	1.3
Average		36.14	36.63	0.49	37.43	0.8	37.83	0.40	38.64	0.81
Total increase in 24 hours:		2.50								
Capon	1	27.7	29.1	1.4	29.2	0.1	29.7	0.5	31.1	1.4
171	2	29.2	29.5	0.3	30.5	1.0	31.2	0.7	33.0	1.8
	3	25.3	26.3	1.0	26.6	0.3	26.6	0.0	28.3	1.7
	4	26.5	27.0	0.5	27.6	0.6	27.9	0.3	29.6	1.7
	5	33.7	34.3	0.6	34.6	0.3	34.6	0.0	36.4	1.8
	6	30.1	31.2	1.1	31.9	0.7	32.0	0.1	32.5	0.5
	7	30.5	31.8	1.3	32.5	0.7	32.6	0.1	33.4	0.8
	8	30.1	30.6	0.5	31.5	0.9	31.5	0.0	32.6	1.1
	9	29.6	30.2	0.6	31.0	0.8	31.4	0.4	32.2	0.8
	10	29.6	30.8	1.2	31.3	0.5	31.5	0.2	32.2	0.7
Average		29.23	30.08	0.85	30.67	0.59	30.9	0.23	32.13	1.23
Total increase in 24 hours:		2.90								
Capon	1	34.6	36.6	2.0	37.1	0.5	37.1	0.0	36.9	-0.2*
139	2	33.6	34.8	1.2	36.2	1.4	36.2	0.0	36.9	0.7
	3	29.2	29.3	0.1	29.9	0.6	29.9	0.0	31.5	1.6
	4	34.1	34.9	0.8	35.5	0.6	35.5	0.0	36.9	1.4
	5	32.4	34.4	2.0	34.6	0.2	34.7	0.1	35.4	0.7
	6	33.2	34.8	1.6	35.0	0.2	35.1	0.1	36.3	1.2
	7	31.5	32.3	0.8	32.3	0.0	32.5	0.2	33.2	0.7
	8	33.6	35.0	1.4	35.3	0.3	35.6	0.3	36.5	0.9
	9	29.6	29.6	0.0	31.4	1.8	31.4	0.0	31.5	0.1
	10	26.5	27.2	0.7	27.6	0.4	27.9	0.3	28.6	0.7
Average		31.83	32.89	1.06	34.49	0.60	33.59	0.10	34.37	0.78
Total increase in 24 hours:		2.54								
GRAND										
AVERAGE		32.40	33.20	0.80	33.86	0.66	34.10	0.24	35.04	0.94
AVERAGE TOTAL INCREASE IN 24 HOURS:		2.64								

\* Probably due to error.

<sup>1</sup> Tables 1 to 8 give all the original measurements for determination of six-hour increments throughout one day, on which the paper is based, together with the averages for





The grand average of the increments in the first set of measurements is plotted in figure 2.

### TABLE 3

TWELVE O'CLOCK BASIS. APRIL 18-19, 1939. SADDLE FEATHERS BROWN LEGHORN  
CAPONS. TWENTY-FIFTH DAY OF REGENERATION

		1 12 NOON	2 6 P. M.	INC.	3 12 P. M.	INC.	4 6 A. M.	INC.	5 12 NOON	INC.
Capon	1	27.4	27.9	0.5	28.8	0.9	28.9	0.1	29.9	1.0
148	2	26.0	26.8	0.8	27.6	0.8	27.7	0.1	28.1	0.4
	3	26.4	26.7	0.3	27.6	0.9	27.6	0.0	28.5	0.9
	4	27.4	28.2	0.8	28.3	0.1	28.4	0.1	30.0	1.6
	5	24.1	24.3	0.2	24.7	0.4	24.7	0.0	26.2	1.5
	6	25.2	25.7	0.5	26.5	0.8	27.0	0.5	27.3	0.3
	7	25.3	25.7	0.4	26.5	0.8	26.6	0.1	27.5	0.9
	8	26.5	27.0	0.5	27.2	0.2	27.2	0.0	27.8	0.6
	9	25.5	25.9	0.4	26.7	0.8	27.0	0.3	27.7	0.7
	10	30.2	30.9	0.7	31.6	0.7	31.7	0.1	32.6	0.9
	Average	26.40	26.91	0.51	27.55	0.64	27.68	0.13	28.56	0.88
	Total increase in 24 hours:			2.16						
Capon	1	27.9	28.2	0.3	29.2	1.0	30.0	0.8	30.5	0.5
171	2	32.7	33.7	1.0	33.9	0.2	34.0	0.1	35.4	1.4
	3	28.0	28.9	0.9	29.3	0.4	29.4	0.1	30.2	0.8
	4	26.8	26.9	0.1	27.3	0.4	27.6	0.3	28.0	0.4
	5	25.5	26.2	0.7	27.0	0.8	27.0	0.0	27.4	0.4
	6	28.2	28.5	0.3	29.1	0.6	29.1	0.0	29.7	0.6
	7	28.1	28.6	0.5	29.1	0.5	29.2	0.1	29.9	0.7
	8	27.0	28.1	1.1	28.7	0.6	28.9	0.2	29.3	0.4
	9	30.4	30.6	0.2	31.0	0.4	31.2	0.2	32.1	0.9
	10	28.4	29.0	0.6	29.8	0.8	29.8	0.0	30.3	0.5
	Average	28.30	28.87	0.57	29.44	0.57	29.62	0.18	30.28	0.66
	Total increase in 24 hours:			1.98						
Capon	1	27.7	28.5	0.8	28.7	0.2	28.7	0.0	29.4	0.7
139	2	27.0	27.4	0.4	28.5	1.1	28.5	0.0	28.6	0.1
	3	29.1	29.3	0.2	30.0	0.7	30.0	0.0	30.9	0.9
	4	28.2	29.0	0.8	29.2	0.2	29.4	0.2	29.9	0.5
	5	27.6	27.8	0.2	29.2	1.4	29.3	0.1	29.9	0.6
	6	27.2	27.9	0.7	28.8	0.9	28.8	0.0	29.9	1.1
	7	24.7	25.8	1.1	26.3	0.5	26.5	0.2	27.4	0.9
	8	27.5	28.5	1.0	29.0	0.5	29.0	0.0	29.6	0.6
	9	28.0	28.6	0.6	29.4	0.8	29.4	0.0	30.2	0.8
	10	26.7	27.1	0.4	27.8	0.7	27.8	0.0	29.1	1.3
	Average	27.37	27.99	0.62	28.69	0.70	28.74	0.05	29.49	0.75
	Total increase in 24 hours:			2.12						
GRAND										
AVERAGE		27.36	27.92	0.56	28.56	0.64	28.68	0.12	29.44	0.76
AVERAGE TOTAL INCREASE IN 24 HOURS:				2.08						



### TABLE 4

TWELVE O'CLOCK BASIS. SECOND SERIES OF MEASUREMENTS OF THE SAME FEATHERS  
AS IN TABLE 3. TWENTY-EIGHTH DAY OF REGENERATION

	1	2		3		4		5		
	12	6		12		6		12		
	NOON	P. M.	INC.	P. M.	INC.	A. M.	INC.	NOON	INC.	
<b>Capon</b>	1	33.7	34.1	0.4	35.1	1.0	35.4	0.3	36.8	1.4
<b>148</b>	2	32.8	33.0	0.2	33.5	0.5	33.8	0.3	34.7	0.9
	3	33.0	33.5	0.5	34.1	0.6	34.3	0.2	34.7	0.4
	4	34.5	35.0	0.5	35.3	0.3	35.3	0.0	36.5	1.2
	5	30.6	31.5	0.9	31.9	0.4	32.5	0.6	33.4	0.9
	6	32.5	32.7	0.2	33.3	0.6	33.3	0.0	34.4	1.1
	7	32.2	32.6	0.4	33.3	0.7	33.4	0.1	33.8	0.4
	8	32.8	33.4	0.6	33.8	0.4	34.4	0.6	34.7	0.3
	9	32.0	32.3	0.3	32.8	0.5	32.8	0.0	33.7	0.9
	10	36.6	37.4	0.8	37.7	0.3	37.8	0.1	38.7	0.9
<b>Average</b>	33.07	33.55	0.48	34.08	0.53	34.30	0.22	35.14	0.84	
<b>Total increase in 24 hours:</b>			2.07							

Capon 171	1	33.3	34.4	1.1	35.6	1.2	35.6	0.0	35.8	0.2
	2	39.0	39.2	0.2	39.6	0.4	39.9	0.3	41.1	1.2
	3	34.0	34.5	0.5	35.3	0.8	35.3	0.0	36.0	0.7
	4	31.3	32.2	0.9	32.6	0.4	32.6	0.0	33.4	0.8
	5	31.4	32.2	0.8	32.6	0.4	32.8	0.2	33.4	0.6
	6	33.5	35.2	1.7	35.5	0.3	35.5	0.0	36.0	0.5
	7	34.1	34.9	0.8	35.5	0.6	35.5	0.0	35.9	0.4
	8	33.5	34.8	1.3	34.9	0.1	35.2	0.3	35.9	0.7
	9	35.5	36.4	0.9	36.8	0.4	36.8	0.0	37.9	1.1
	10	34.0	34.7	0.7	35.3	0.6	35.3	0.0	36.0	0.7
Average		33.96	34.85	0.89	35.37	0.52	35.45	0.08	36.14	0.69
Total increase in 24 hours:				2.18						

Capon 139	1	33.5	34.7	1.2	34.9	0.2	35.2	0.3	35.5	0.3
	2	31.2	32.0	0.8	33.0	1.0	33.0	0.0	33.4	0.4
	3	35.1	35.5	0.4	36.5	1.0	36.9	0.4	37.5	0.6
	4	32.1	32.3	0.2	32.8	0.5	32.9	0.1	33.2	0.3
	5	33.2	33.9	0.7	35.0	1.1	35.0	0.0	35.4	0.4
	6	33.1	33.9	0.8	35.0	1.1	35.1	0.1	35.7	0.6
	7	30.8	31.4	0.6	31.7	0.3	32.0	0.3	32.9	0.9
	8	33.7	34.6	0.9	34.7	0.1	34.7	0.0	35.2	0.5
	9	34.2	34.8	0.6	35.3	0.5	35.3	0.0	35.8	0.5
	10	32.7	33.4	0.7	33.9	0.5	33.9	0.0	34.8	0.9
Average		32.96	33.65	0.69	34.28	0.63	34.40	0.12	34.94	0.54
Total increase in 24 hours:				1.98						

GRAND									
AVERAGE	33.33	34.02	0.69	34.58	0.56	34.72	0.14	35.41	0.69
AVERAGE TOTAL INCREASE IN 24 HOURS:	2.08								

Measurements were made to the nearest tenth of a millimeter as follows: A piece of white paper was inserted beneath the feather which was then held flat against it, and an original measurement was made with spring nut outside calipers from the mouth of the follicle on the upper side to the tip of the feather. The space between the points of the spring calipers was then measured with vernier calipers with direct gauge reading to 0.1 mm. (made by Schietrumpf of Jena). The part of the feather within the follicle (about 7 mm. in length) was not taken into account. The principal sources of error by this method are, first, that the position of the points of the spring calipers in the original measurements must be judged by the eye alone, and, second, the assumption is made that the opening of the follicle maintains a fixed position during the 24 hours. These possible sources of error tend to be averaged out. The very large averaged difference between the six-hour period from midnight to 6:00 A. M. and the other six-hour measurements are consistent for the separate measurements with some exceptions (see tables).

The average growth in 24 hours of all breast feathers in both sets of determinations was 2.58 mm.; for all saddle feathers, 2.08 mm. If the growth in each six-hour period in the diurnal cycle were uniform, each would record 25% of this amount. The actual percentages,<sup>1</sup> averaging all determinations for each six-hour period for breast and for saddle, were as follows:

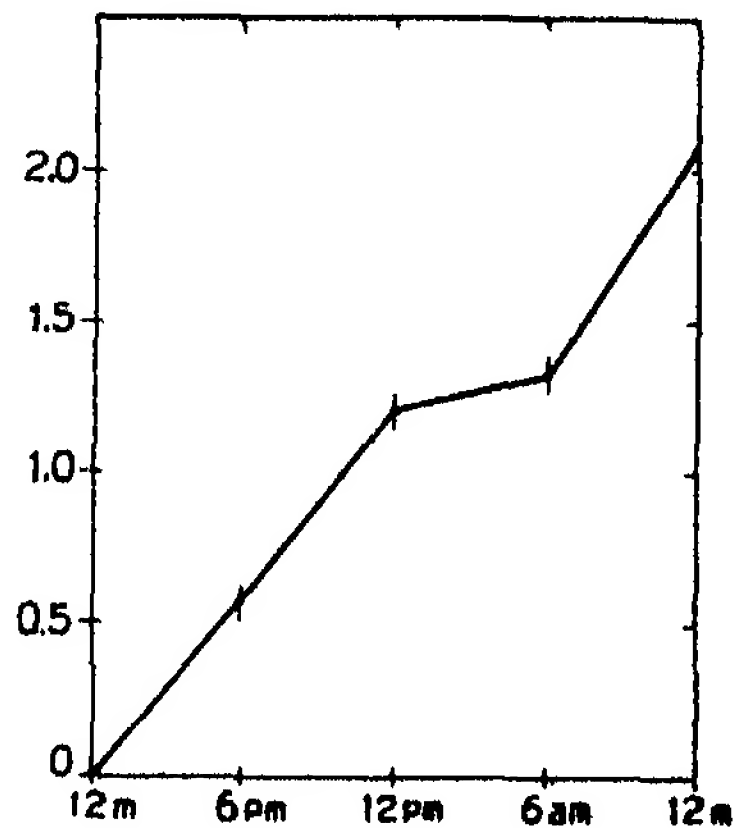


FIGURE 2

Saddle feathers. From data of table 3. The grand average of six-hour increments of the three birds plotted. Based on 12:00 o'clock. Ordinates = millimeters of growth; abscissae = time of day.

	BREAST	SADDLE
12:00 noon to 6:00 P. M.	31%	30%
6:00 P. M. to 12:00 midnight	26%	29%
12:00 midnight to 6:00 A. M.	9%	6%
6:00 A. M. to 12:00 noon	34%	35%

It will be noted that the amount of depression of growth rate from midnight to 6:00 A. M. is greater in the saddle than in the breast. This is perhaps due to a lower threshold of susceptibility similar to that which Juhn and Gustavson ('30) found in saddle as compared with breast feathers

<sup>1</sup> The nearest whole number is used for each percentage.

in the case of reaction to the female hormone, and which they attributed to slower rate of growth.

It is interesting to note that 50 zero increments are recorded between midnight and 6:00 A. M. out of the 120 records made for this period, only one between noon and 6:00 P. M., two between 6:00 P. M. and midnight and none between 6:00 A. M. and noon, similarly out of 120 records for each period. Two of the determinations for six-hour periods are small decrements, 0.1 and 0.2, respectively, obviously due to errors of recording. There are five records between midnight and 6:00 A. M. out of 120 in

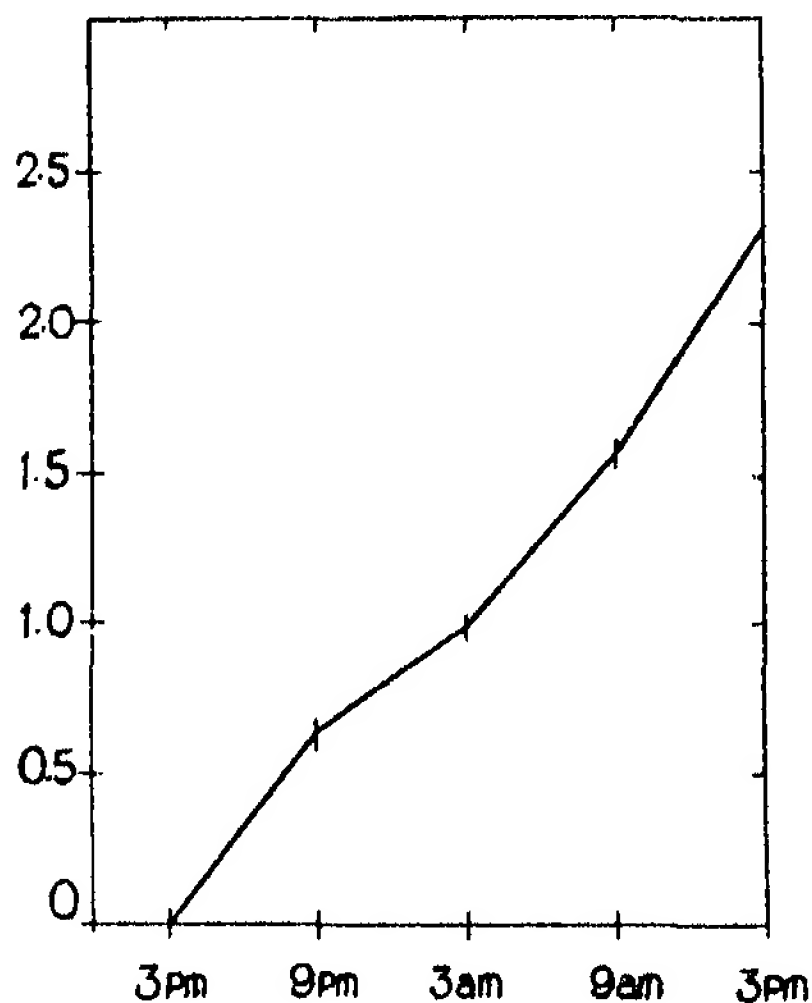


FIGURE 3

Breast feathers. From data of table 5. The grand average of six-hour increments of the three birds plotted. Based on 9:00 o'clock. Ordinates = millimeters of growth; abscissae = time of day.

which the increment of individual feathers exceeds 25% of the total growth for 24 hours. The range of variation of increment within each period is considerable. There are also slight differences to be noted between the three birds used.

Except for the disturbance due to measurement, the birds were on a normal régime with no artificial lighting. No attempt has been made to determine how differences in feeding, lighting or activity would affect the diurnal cycle.

2. *Six-Hour Increments Based on Nine O'Clock.*—The amount of growth was then measured at six-hour periods beginning at nine o'clock instead of twelve o'clock. The regeneration age at the beginning of the measurements was 20 days in the case of the breast feathers and 22 days in the case of the saddle feathers. The same

three capons were used as before, and precisely the same methods were followed. The observations were made June 12–14 for the breast feathers (tables 5 and 6 and Fig. 3), and June 24–26 for the saddle feathers (tables 7 and 8 and Fig. 4). In each case two consecutive 24-hour periods were used. In the case of the measurements beginning at twelve o'clock, the two 24-hour periods were separated by three days in both breast and saddle feathers. The measurements for six-hour periods beginning at twelve o'clock were made in March and April when the temperature of the animal house was much lower than in the case of the periods based on nine o'clock, which were made in June. These differences may have had some effect.

TABLE 5

NINE O'CLOCK BASIS. JUNE 12-13, 1939. BREAST FEATHERS BROWN LEGHORN  
CAPONS. TWENTIETH DAY OF REGENERATION

		1	2			3	4			5		
		9	3			9	3			9		
		A. M.	P. M.			P. M.	INC.			A. M.		
Capon 148	1	20.3	21.1	0.8	21.7	0.6	22.0	0.3	22.1	0.1		
	2	20.0	20.7	0.7	20.9	0.2	21.5	0.6	22.0	0.5		
	3	20.1	21.5	1.4	22.2	0.7	22.2	0.0	22.4	0.2		
	4	19.7	20.5	0.8	21.4	0.9	21.4	0.0	22.2	0.8		
	5	18.9	19.8	0.9	21.2	1.4	21.3	0.1	22.0	0.7		
	6	18.0	18.8	0.8	19.5	0.7	20.2	0.7	20.7	0.5		
	7	18.0	18.8	0.8	19.5	0.7	20.5	1.0	21.3	0.8		
	8	18.9	19.7	0.8	20.4	0.7	20.8	0.4	21.3	0.5		
	9	18.7	19.8	1.1	20.4	0.6	20.8	0.4	21.3	0.5		
	10	16.7	17.7	1.0	17.9	0.2	18.3	0.4	19.1	0.8		
Average		18.93	19.84	0.91	20.51	0.67	20.90	0.39	21.44	0.54		
Total increase in 24 hours:				2.51								
Capon 171	1	16.6	17.4	0.8	18.0	0.6	18.0	0.0	18.7	0.7		
	2	16.6	17.4	0.8	18.0	0.6	18.4	0.4	19.4	1.0		
	3	16.0	16.5	0.5	17.0	0.5	17.5	0.5	18.0	0.5		
	4	15.0	15.9	0.9	16.3	0.4	16.9	0.6	17.6	0.7		
	5	15.5	16.1	0.6	17.2	1.1	17.6	0.4	17.9	0.3		
	6	15.2	15.8	0.6	16.7	0.9	17.6	0.9	17.7	0.1		
	7	15.0	15.6	0.6	15.9	0.3	16.5	0.6	16.7	0.2		
	8	15.8	16.8	1.0	17.4	0.6	17.4	0.0	18.3	0.9		
	9	15.6	16.7	1.1	17.6	0.9	17.9	0.3	18.4	0.5		
	10	13.7	14.6	0.9	15.3	0.7	15.3	0.0	16.4	1.1		
Average		15.50	16.28	0.78	16.94	0.66	17.31	0.37	17.91	0.60		
Total increase in 24 hours:				2.41								
Capon 139	1	20.2	20.6	0.4	20.8	0.2	20.9	0.1	21.5	0.6		
	2	20.3	21.1	0.8	21.5	0.4	21.6	0.1	21.9	0.3		
	3	17.5	18.1	0.6	18.6	0.5	18.9	0.3	19.3	0.4		
	4	21.1	21.7	0.6	22.2	0.5	22.3	0.1	23.1	0.8		
	5	19.7	20.6	0.9	21.5	0.9	21.5	0.0	21.9	0.4		
	6	21.0	21.5	0.5	22.1	0.6	22.9	0.8	23.3	0.4		
	7	19.7	20.4	0.7	20.9	0.5	21.4	0.5	21.8	0.4		
	8	20.7	21.2	0.5	21.9	0.7	22.9	1.0	23.3	0.4		
	9	19.7	20.0	0.3	20.8	0.8	20.9	0.1	21.7	0.8		
	10	18.9	19.4	0.5	19.8	0.4	20.1	0.3	21.2	1.1		
Average		19.88	20.46	0.58	21.01	0.55	21.34	0.33	21.90	0.56		
Total increase in 24 hours:				2.02								
GRAND												
AVERAGE		18.10	18.86	0.76	19.49	0.63	19.85	0.36	20.42	0.57		
AVERAGE TOTAL INCREASE IN 24 HOURS:				2.32								

TABLE 6

NINE O'CLOCK BASIS. SECOND SERIES OF MEASUREMENTS OF THE SAME FEATHERS AS IN TABLE 5. TWENTY-FIRST DAY OF REGENERATION

		1	2			3	4			5		
		9	3			9	3			9		
		A. M.	P. M.			P. M.	INC.			A. M.		
Capon 148	1	22.1	22.8	0.7	23.3	0.5	23.5	0.2	24.7	1.2		
	2	22.0	22.4	0.4	22.8	0.4	23.5	0.7	24.5	1.0		
	3	22.4	23.4	1.0	23.9	0.5	24.2	0.3	25.1	0.9		
	4	22.2	22.5	0.3	23.3	0.8	23.4	0.1	24.2	0.8		
	5	22.0	22.4	0.4	23.2	0.8	23.5	0.3	24.2	0.7		
	6	20.7	21.4	0.7	21.9	0.5	22.6	0.7	23.3	0.7		
	7	21.3	21.7	0.4	22.7	1.0	23.2	0.5	23.7	0.5		
	8	21.3	22.2	0.9	22.7	0.5	23.2	0.5	24.0	0.8		
	9	21.3	22.2	0.9	22.7	0.5	23.0	0.3	24.1	1.1		
	10	19.1	20.0	0.9	20.2	0.2	21.0	0.8	21.6	0.6		
Average		21.44	22.10	0.66	22.67	0.57	23.11	0.44	23.94	0.83		
Total increase in 24 hours:				2.50								
Capon 171	1	18.7	19.8	1.1	20.1	0.3	20.8	0.7	21.5	0.7		
	2	19.4	20.5	1.1	20.8	0.3	21.5	0.7	21.6	0.1		
	3	18.0	18.6	0.6	19.2	0.6	20.0	0.8	20.5	0.5		
	4	17.6	18.5	0.9	18.8	0.3	19.4	0.6	20.0	0.6		
	5	17.9	19.0	1.1	19.6	0.6	20.4	0.8	20.8	0.4		
	6	17.7	18.5	0.8	19.3	0.8	19.6	0.3	20.0	0.4		
	7	16.7	17.6	0.9	18.5	0.9	18.8	0.3	19.5	0.7		
	8	18.3	19.0	0.7	19.8	0.8	20.2	0.4	20.9	0.7		
	9	18.4	19.5	1.1	20.2	0.7	20.7	0.5	21.5	0.8		
	10	16.4	17.2	0.8	17.5	0.3	18.0	0.5	18.6	0.6		
Average		17.91	18.82	0.91	19.38	0.56	19.94	0.56	20.49	0.55		
Total increase in 24 hours:				2.58								
Capon 139	1	21.5	22.5	1.0	23.1	0.6	23.7	0.6	24.3	0.6		
	2	21.9	22.8	0.9	23.5	0.7	23.7	0.2	24.3	0.6		
	3*	18.9	19.6	0.7	20.0	0.4	20.4	0.4	21.6	1.2		
	4	23.1	23.7	0.6	24.4	0.7	24.8	0.4	25.4	0.6		
	5	21.9	22.3	0.4	22.9	0.6	23.4	0.5	23.8	0.4		
	6	23.3	24.1	0.8	24.7	0.6	25.4	0.7	25.6	0.2		
	7	21.8	22.4	0.6	23.2	0.8	23.5	0.3	24.3	0.8		
	8	23.3	23.5	0.2	24.3	0.8	24.6	0.3	25.7	1.1		
	9	21.7	22.5	0.8	22.8	0.3	23.4	0.6	24.5	1.1		
	10	21.2	21.5	0.3	22.0	0.5	22.4	0.4	23.5	1.1		
Average		21.86	22.49	0.63	23.09	0.60	23.53	0.44	24.30	0.77		
Total increase in 24 hours:				2.44								
GRAND												
AVERAGE		20.40	21.13	0.73	21.71	0.58	22.19	0.48	22.91	0.72		
AVERAGE TOTAL INCREASE IN 24 HOURS:				2.51								

\* A newly selected feather to replace the one used in the first series of measurements (table 5) which was lost.

### TABLE 7

NINE O'CLOCK BASIS. JUNE 24-25, 1939. SADDLE FEATHERS BROWN LEGHORN  
CAPONS. TWENTY-SECOND DAY OF REGENERATION

		1	2		3		4		5	
		9	3		9		3		9	
		A. M.	P. M.	INC.	P. M.	INC.	A. M.	INC.	A. M.	INC.
Capon	1	27.7	28.2	0.5	28.7	0.5	28.8	0.1	29.3	0.5
148	2	17.4	17.9	0.5	18.2	0.3	18.7	0.5	19.1	0.4
	3	17.8	18.2	0.4	18.7	0.5	18.8	0.1	19.2	0.4
	4	18.9	19.5	0.6	19.7	0.2	19.9	0.2	20.6	0.7
	5	12.3	13.2	0.9	13.6	0.4	14.0	0.4	14.7	0.7
	6	14.3	15.2	0.9	15.9	0.7	15.9	0.0	16.3	0.4
	7	14.1	14.8	0.7	15.1	0.3	15.2	0.1	15.8	0.6
	8	14.1	14.8	0.7	15.1	0.3	15.5	0.4	16.2	0.7
	9	14.9	15.2	0.3	15.8	0.6	16.2	0.4	16.7	0.5
	10	16.1	16.4	0.3	16.7	0.3	16.9	0.2	17.3	0.4
	Average	16.76	17.34	0.58	17.75	0.41	17.99	0.24	18.52	0.53
	Total increase in 24 hours:			1.76						
Capon	1	17.8	18.4	0.6	18.9	0.5	19.1	0.2	19.6	0.5
171	2	18.4	19.0	0.6	19.5	0.5	19.6	0.1	20.2	0.6
	3	17.5	17.8	0.3	18.5	0.7	18.7	0.2	19.3	0.6
	4	16.6	16.9	0.4	17.4	0.5	17.6	0.2	18.2	0.6
	5	18.4	18.7	0.3	19.3	0.6	19.5	0.2	20.1	0.6
	6	18.7	19.0	0.3	19.5	0.5	19.6	0.1	20.5	0.9
	7	15.5	16.2	0.7	16.8	0.6	17.2	0.4	17.5	0.3
	8	18.6	19.4	0.8	19.8	0.4	20.1	0.3	20.4	0.3
	9	17.5	18.4	0.9	18.6	0.2	18.7	0.1	19.7	1.0
	10	16.8	17.5	0.7	18.0	0.5	18.4	0.4	18.8	0.4
	Average	17.57	18.13	0.56	18.63	0.50	18.85	0.22	19.43	0.58
	Total increase in 24 hours:			1.86						
Capon	1	15.7	16.0	0.3	17.0	1.0	17.3	0.3	17.9	0.6
139	2	14.7	15.3	0.6	15.9	0.6	16.0	0.1	16.6	0.6
	3	24.5	25.1	0.6	25.4	0.3	25.8	0.4	26.3	0.5
	4	15.5	16.0	0.5	16.7	0.7	17.0	0.3	17.6	0.6
	5	15.5	16.1	0.6	16.5	0.4	16.8	0.3	17.3	0.5
	6	17.1	17.5	0.4	18.3	0.8	18.4	0.1	19.1	0.7
	7	17.8	18.5	0.7	18.8	0.3	19.3	0.5	19.9	0.6
	8	14.6	15.5	0.9	16.0	0.5	16.3	0.3	16.7	0.4
	9	14.8	15.8	1.0	16.1	0.3	16.3	0.2	16.7	0.4
	10	17.2	17.8	0.6	18.1	0.3	18.5	0.4	19.1	0.6
	Average	16.74	17.36	0.62	17.88	0.52	18.17	0.29	18.72	0.55
	Total increase in 24 hours:			1.98						
GRAND										
AVERAGE		17.02	17.61	0.59	18.09	0.48	18.34	0.25	18.89	0.55
AVERAGE TOTAL INCREASE IN 24 HOURS:				1.87						

GRAND									
AVERAGE	18.89	19.49	0.60	20.06	0.57	20.36	0.30	20.98	0.62
AVERAGE TOTAL INCREASE IN 24 HOURS: 2.09									



The average growth in 24 hours of all breast feathers in both sets of determinations was 2.15 mm., of all saddle feathers, 1.98 mm., in each case somewhat less than the previous sets. The averages of percentages of daily increments for six-hour periods were as follows:

	BREAST		SADDLE	
	1ST DAY	2ND DAY	1ST DAY	2ND DAY
9:00 A. M. to 3:00 P. M.	32.76	29.08	31.55	28.70
3:00 P. M. to 9:00 P. M.	27.15	23.11	25.67	27.27
9:00 P. M. to 3:00 A. M.	15.52	19.12	13.37	14.36
3:00 A. M. to 9:00 A. M.	24.57	28.69	29.41	29.67

There is a depression of growth rate here from 9:00 P. M. to 3:00 A. M. but of a lesser order of magnitude than from 12:00 midnight to 6:00 A. M. in the former series (cf. Figs. 1 to 4).

### 3. Comparison of the Twelve O'Clock and Nine O'Clock Bases.—

Growth within each six-hour period is necessarily represented as uniform owing to the requirements of the method; but it is obviously improbable in the highest degree that this is actually the case. The purpose of repeating the original observations made on a twelve o'clock basis by another set of observations based on nine o'clock was to ascertain whether by comparison it would be possible to determine at least the three-hour period with the lowest rate of growth. The remainder of the diurnal curves of growth (cf. Figs. 1 to 4) does not exhibit sufficient variation to encourage an attempt at closer determination of the time of maximum rate of growth.

In table 9 we have divided the growth of each six-hour period into equal halves, thus establishing arbitrary three-hour periods of growth both on the twelve o'clock and the nine o'clock bases; these figures are taken from the grand averages of the first set of measurements in each case, and the corresponding three-hour periods for the two bases are thus brought directly into comparison. It will be seen that except for the periods of depression of growth rates, 12:00 P. M. to 6:00 A. M. on the twelve o'clock basis, and 9:00 P. M. to 3:00 A. M. on the nine o'clock basis, the three-hour periods

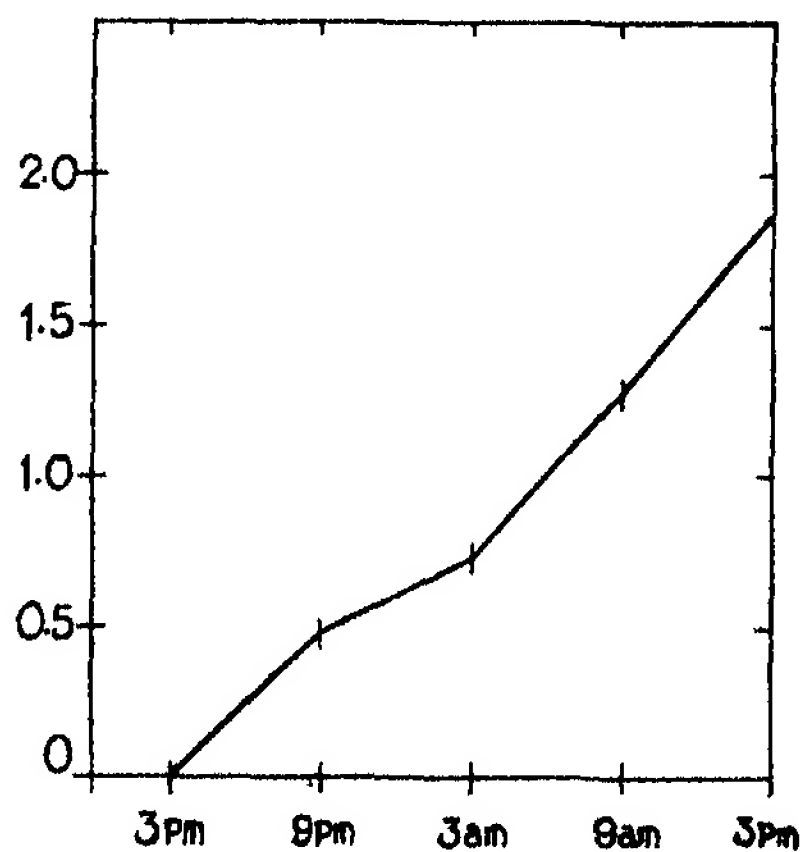


FIGURE 4


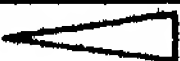

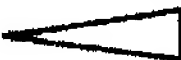
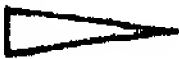
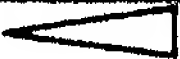
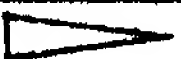

Saddle feathers. From data of table 7. The grand average of six-hour increments of the three birds plotted. Based on 9:00 o'clock. Ordinates = millimeters of growth; abscissae = time of day.

agree fairly well in the two series. In spite of the great discrepancies between the twelve o'clock and the nine o'clock bases during the periods of depression, it is nevertheless shown by the table that the sum of the amount of growth on the two bases in the three-hour period from 12:00 P. M. to 3:00 A. M. is much less than in any other three-hour period, even comparing the two adjacent three-hour periods. We would therefore conclude that the period of least growth is from midnight to 3:00 A. M.

This reinforces the conclusion that the diurnal curve of growth is in reality a flowing, and not an angular, curve. We have attempted to apply this conception by the triangular signs in table 9. These are intended to indicate that on the twelve o'clock basis the growth from 6:00 P. M. to 9:00 P. M. is probably more than from 9:00 P. M. to midnight, and that the

TABLE 9

DATA REARRANGED ON THE BASIS OF THREE-HOUR INCREMENTS. ALL DATA BASED ON THE AVERAGE OF THE FIRST SET OF MEASUREMENTS OF ALL THE THREE BROWN LEGHORN CAPONS USED

	12 M.- 3 P. M.	3 P. M.- 6 P. M.	6 P. M.- 9 P. M.	9 P. M.- 12 P. M.	12 P. M.- 3 A. M.	3 A. M.- 6 A. M.	6 A. M.- 9 A. M.	9 A. M.- 12 M.	TOTAL INC.
<b>Breast</b>									
12 o'clock basis	0.40	0.40	0.33	0.33	0.12	0.12	0.47	0.47	2.64 mm.
									
9 o'clock basis	0.38	0.315	0.315	0.18	0.18	0.285	0.285	0.38	2.32 mm.
									
<b>Saddle</b>									
12 o'clock basis	0.28	0.28	0.32	0.32	0.06	0.06	0.38	0.38	2.08 mm.
									
9 o'clock basis	0.295	0.24	0.24	0.125	0.125	0.275	0.275	0.295	1.87 mm.
									

growth from midnight to 3:00 A. M. is probably less than from 3:00 A. M. to 6:00 A. M. Similarly, on the nine o'clock basis the growth from 9:00 P. M. to midnight is probably more than from midnight to 3:00 A. M., and the growth from 3:00 A. M. to 6:00 A. M. less than from 6:00 A. M. to 9:00 A. M. Any corrections made in this sense would tend to even out the discrepancies between the two bases.

In figures 5 and 6 we have applied the principle of these corrections to the curves of growth for the breast feathers on the twelve o'clock and nine o'clock bases respectively (Figs. 1 and 2). The corrections are indicated by the flowing dotted curves crossing the original curves. The exact form of the dotted curve represents merely our best judgment of the amount and distribution of the corrections, and to that extent is arbitrary, but it is

believed to give a more correct representation of the actual growth rate than either of the unmodified curves. The six-hour period of least growth is emphasized by the stippled triangle below it. The 3:00 A. M. ordinate divides it into unequal parts.

### III. DISCUSSION

1. *The Physiological Basis of the Diurnal Rhythm.*—(a) Diurnal fluctuations of basal metabolism. The determinations of Barott and others ('38)<sup>1</sup> of the diurnal fluctuations of the energy production and gaseous metabolism of male Rhode Island chickens aged from four to 130 days are by far the most thorough on record. On page 158 they give a curve of the diurnal rhythm of the energy metabolism, as measured by oxygen consumption, of fasting male Rhode Island Red chickens of between 15 and about 18 weeks of age. This is a very regular flowing curve covering three days. The high point of the curve occurs daily between 8:00 A. M. and 9:00 A. M. and the low point between 8:00 P. M. and 9:00 P. M. On the second day of the three-day record, the high oxygen consumption was about 0.85 cc. per hour per gram live weight, and the low about 0.72.

Bacq ('29) studied four cocks both under normal feeding and fasting régimes. His curve of diurnal fluctuations of basal metabolism (calories per kg. hr.) is quite similar to those of Barott though the observations are rather fragmentary; the low and high points are slightly later.

If we attempt a closer comparison to the oxygen consumption curve of Barott *et al.* we find that the lowest three-hour period of oxygen consumption, which offers the best basis for comparison of growth in the 24-hour cycle, is, according to their determinations, between 6:00 P. M. and 9:00 P. M. approximately, whereas the period of least growth according to our determinations is between midnight and 3:00 A. M., thus six hours later.

<sup>1</sup> Cf. also Benedict, Landauer and Fox ('32), and Scharnke ('32).

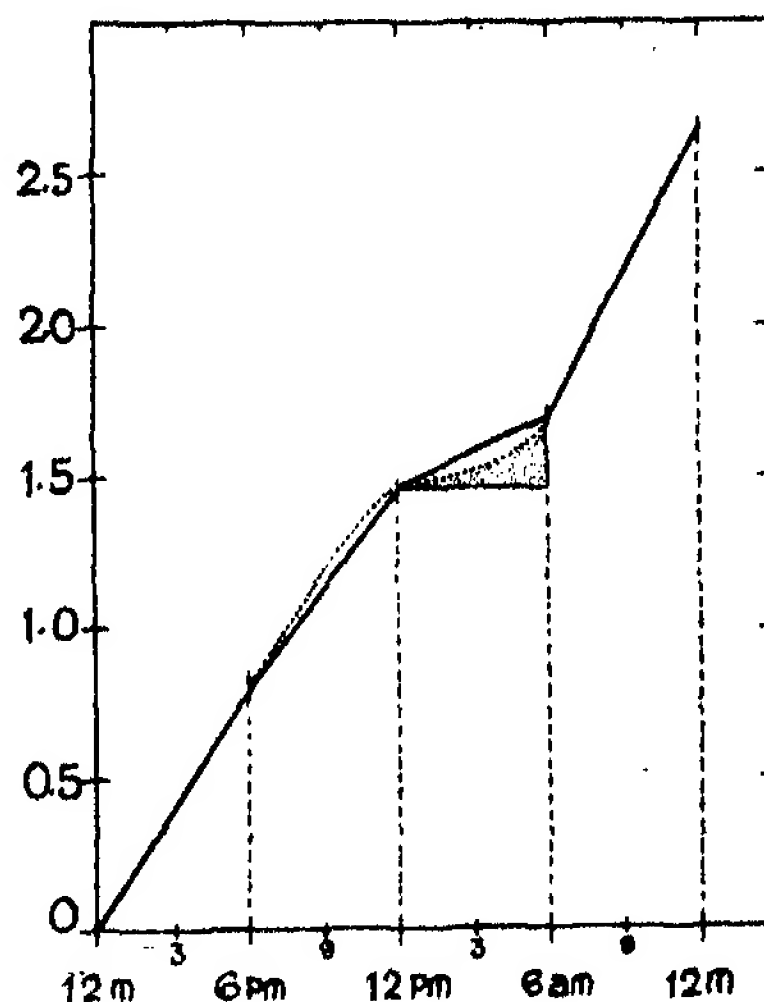


FIGURE 5

Breast feathers. The 12:00 o'clock basis (Fig. 1) modified by the dotted line based on comparison of the 9:00 o'clock basis (Fig. 3) as explained in table 9. The stippled area emphasizes the period of the least six-hour increment. Ordinates = millimeters of growth; abscissae = time of day.

If we assume that there is a causal relation between energy metabolism and growth in length of the feather, we have to find some explanation of the very considerable lag of effect.

Lillie and Juhn ('32) attempted to estimate the growth due to plasmatic growth, including cell-division, and to cell-differentiation, respectively, in the growth in length of the shaft (p. 143) and in the barbs (p. 150), and concluded that about 90% of growth in length is due to cell-differentiation and only about 10% to plasmatic growth. The amount of growth of the whole feather due to cell-differentiation, which consists, so far as magnitudes are concerned, in elongation and "ballooning" of cells, depends on the number of cells presented for differentiation as presumably the most important factor; and the number presented at any one time will depend on the rate of cell-division.

Even if the rate of cell-division should respond immediately to reduced  $O_2$  in the blood, time would be required for the new cells to move up into the zone of differentiation, and before reduction in the number of cells available for differentiation becomes a measurable factor in reduction of rate of growth. It is therefore not to be expected that the dampening effects of lower metabolism will receive simultaneous response in measurements of length increments at six-hour intervals. A certain amount of lag is to be expected on the hypothesis of a causal relation between the rates of energy metabolism and growth increments in such a system as the regenerating feather.

(b) Diurnal fluctuations of body temperature. Regular diurnal fluctuations of internal body temperature have often been recorded. Simpson and Galbraith ('05) record a maximum temperature of  $41.9^{\circ}C$ . for Dorking males at 3:00 P. M. and a minimum temperature of  $40.9^{\circ}$  at 3:00 A. M. The temperature drops suddenly from 6:00 P. M. to 9:00 P. M. and then holds near, or below,  $41^{\circ}$  to about 4:00 A. M. Riddle ('07) similarly recorded a marked drop in temperature in fowl, ducks and pigeons during the night. Hilden and Stenback ('16) record a series of observations on fowl and eight other species of birds confirming in general the determinations of Simpson and Galbraith, but adding the valuable determination that the regular day and night variations are readily reversed by keeping the birds in the dark during the day and furnishing illumination at night. A more systematic study of the diurnal temperature fluctuations in fowl under various conditions is, however, still much to be desired.

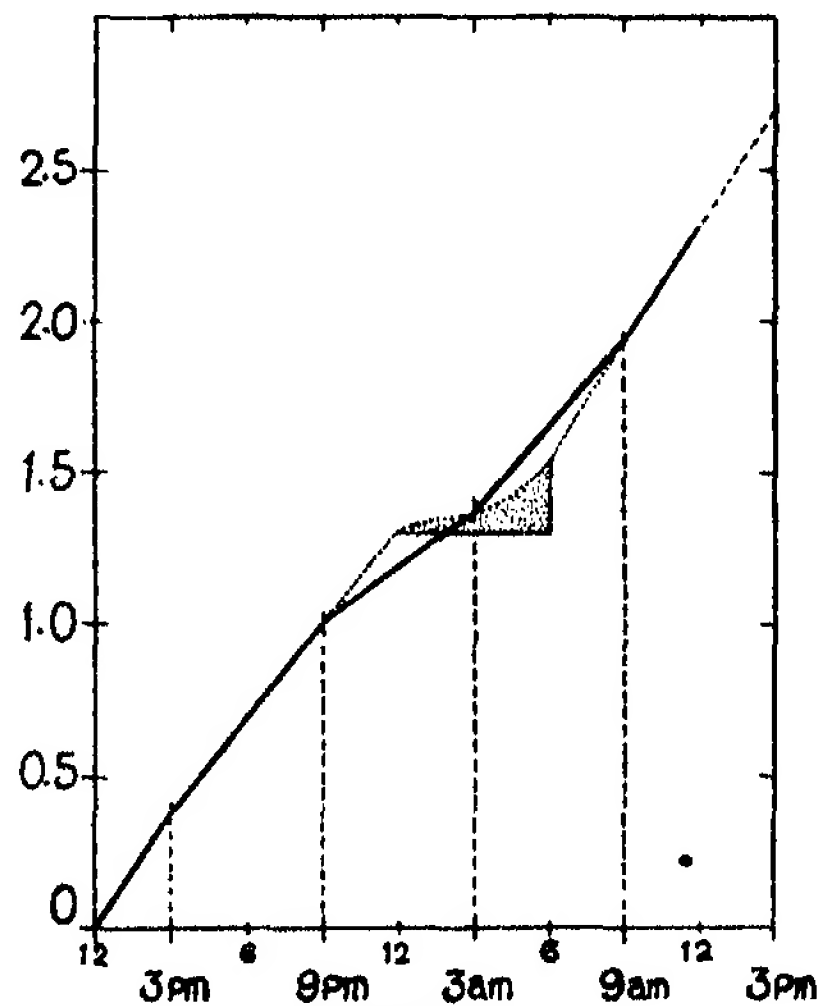
As regards other birds, Simpson and Galbraith ('05) record a much greater depression of body temperature during the night than in fowl in a considerable number of species, especially smaller birds; but they found that in the case of the owl, a bird of nocturnal habit, the situation was reversed. Wetmore ('21) made an immense number of careful determinations of body temperatures of birds taken in the field, and found, as regards

diurnal variations, marked depressions during the night, with the exception of birds of nocturnal habit in which the situation was reversed, confirming Simpson and Galbraith.

Riley ('37) reports daily variation in the body temperature of the house sparrow ranging from an average of  $110^{\circ}\text{F}$ . during the day to  $103\text{--}104^{\circ}\text{F}$ . during the night. Spermatogenetic activity is limited to the period of reduced body temperature. Artificial inversion of light and dark periods with reference to normal day and night inverts also the normal day and night body temperatures and the time of spermatogenetic activity.

Huff ('39) has made a study of diurnal variation of temperature in canaries in connection with his study of malarial infections. He made several series of temperature readings every three hours in females by inserting hypodermic thermocouples deep into the pectoral muscles. The mean day temperature for all determinations was  $42.3^{\circ}\text{C.} \pm 0.05$ , and the mean night temperature  $41.0^{\circ}\text{C.} \pm 0.26$ . In certain apparently normal individuals the range might be as much as  $6^{\circ}\text{C.}$  over a 24-hour period. The curves show a sharp drop of temperature beginning about 4:00 P. M., reaching its lowest point about 3:00 A. M. to 4:00 A. M. and then sharply rising.

In attempting to compare the diurnal curve of growth with that of internal body temperature in fowl, we are limited by the lack of continuous records of the latter under controlled conditions. Simpson and Galbraith ('05) record almost a plateau of low temperature from 9:00 P. M. to 3:00 A. M. from observations of a single male and female Dorking fowl. In canaries (Huff, '39) the high daytime body temperature begins to fall in the late afternoon and reaches its lowest point about 3:00 A. M. to 4:00 A. M. and then rises sharply. If it is permissible to combine observations on temperature in different species of birds for comparison with our growth rate on fowls we note that the rather sudden rise in body temperature about 3:00 A. M. to 4:00 A. M. agrees well with the sudden increase of rate



Breast feathers. The 9:00 o'clock basis (Fig. 3) modified by the dotted line based on comparison of the 12:00 o'clock basis (Fig. 1) as explained in table 9. The stippled area emphasizes the period of the least six-hour increment. Ordinates = millimeters of growth; abscissae = time of day.



of growth of the feather at the same time, and the association of the regularly occurring night depression of body temperature and growth must be held to be significant. Precise comparisons would require simultaneous determinations of body temperature and rate of growth of feathers under carefully controlled conditions.

It would seem to be indicated that daily fatigue followed by rest involves a decrease of the rate of basal metabolism, which causes a drop in body temperature associated with a reduction in rate of growth of the regenerating feather. The fact that day-night body temperature relations are inverted in nocturnal birds would seem to indicate that light as such does not play a direct rôle in the presumed sequence of events.

2. *Morphological Consequences of the Diurnal Rhythm.*—The determination that the rate of growth undergoes a very sharp diminution each 24 hours between midnight and 3:00 A. M. checks well with C. O. Whitman's discovery of the fundamental bars of feathers, which have been especially studied by Riddle ('07, '08). Riddle believed, on the basis of good evidence, that each bar represents a single day of growth, and that the "defective lines, or points of apposition of the fundamental bars," are the loci of formation of the abnormal fault bars, characterized by absence or defect of barbules, which occasionally cross the vane of feathers at a constant angle. The fault bars were subsequently studied by Fraps and Juhn ('36) and interpreted as isochrones, i.e., as lines of simultaneous disturbance of development in the germ, in their entire extent, thus referable to a cause acting at one time.

H. and Josephine Michener ('38) have described bars that occur in flight feathers of house finches also which they studied during regeneration after plucking. They conclude that the bars across the feathers, resembling watermarks in paper, represent a single day's growth. They find that they are most distinct in winter-grown feathers and surmise that they are due to difference in metabolism at night and in the day.

It is indeed probable that the fundamental bars of feathers are related to diurnal physiological rhythms, but this remains at present hypothetical. The occurrence of such considerable depression of the growth rate of developing feathers during the night as we have determined for fowl offers a better mode of attack on this problem; for it has been established that thresholds of reaction in developing feathers vary according to rate of growth (Juhn and Gustavson, '32; Juhn, Faulkner and Gustavson, '31); moreover, Lillie and Juhn ('32, p. 170) have shown that component parts of the same feather, such as barbules and melanophores, have differential thresholds. On such a basis it should be possible to investigate more precisely than has hitherto been done the fundamental bars and other possible morphological consequences of diurnal rhythms in feathers.

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## THE PIGMENT-FORMING POTENCY OF EARLY CHICK BLASTODERMS

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Recent transplantation experiments<sup>2,3,8</sup> between various breeds of fowl have clearly established the origin of melanophores in the chick from the neural crest. According to Holmdahl<sup>4</sup> this structure first appears in the midbrain region at the 6 somite stage. By this time the greater portion of the medullary plate has been converted already into the neural tube and the three primary regions of the brain differentiated. The question comes up as to where the cells with pigment-forming potency are located before there is any definite morphological indication of the neural crest. To answer this the present experiments were undertaken. Small measured pieces, on the average 0.5 mm. square, were isolated from blastoderms varying in development from the unincubated stage to 8 somites, and transplanted to the right wing bud region of 70–80 hr. host embryos of a different breed. For a description of the methods of cutting and handling small pieces of early blastoderms and grafting to the wing bud, the reader is referred to former publications.<sup>6,7,11</sup>

In the majority of cases the donors were Barred Plymouth Rocks and the host White Leghorns, but Black Minorcas and a  $F_1$  hybrid black breed (Barred Plymouth Rock ♀ × Rhode Island Red ♂) also served as donors, and New Hampshire Reds and White Wyandottes as hosts.<sup>12</sup>

A total of 185 operated host embryos lived to attain full down plumage and were examined for pigment formation in the feather cells. Of these 74 (40%) actually hatched, the great majority of which lived to be adults and were followed through their various plumage changes.

*The Experiments.*—a. *Donors 1–8 somites.* Transplants from Barred Plymouth Rock donor blastoderms which included portions of the neural tube, brain wall and Hensen's node or regions not more than 0.3 mm. lateral and posterior to it, produced extensive areas of black down feathers on White Leghorn hosts covering the wing (cf. Fig. 2) and often adjacent regions of breast and back. Transplants from all other regions, the primitive streak, somites, lateral and anterior portions of the area pellucida gave negative results, i.e., produced no black color in the down feathers of the White Leghorn hosts. In donors of 6, 7 and 8 somites, no transplants were taken from the midbrain region in order to be sure to exclude neural crest cells which at this time are beginning to proliferate there.

b. *Head-process donors.* To test the capacity of all regions of the blastoderm at various stages of head-process development for pigment pro-

duction, the entire area pellucida was divided by means of transverse and longitudinal cuts into a number of small pieces (Fig. 1) and each transplanted separately to the wing bud of a host embryo. Record was kept of the original position of each piece with reference to the primitive pit, which marks the center of the node region and is a convenient point from which to take measurements. Blastoderms of Barred Plymouth Rock were tested on White Leghorn and New Hampshire Red hosts, Black Minorca on White



FIGURE 1

Photomicrograph of a living donor blastoderm of the definitive primitive streak stage divided into a number of small measured pieces preparatory to transplantation. See text. ( $\times 10$ .)

Leghorn and  $F_1$  hybrid black on White Leghorn and White Wyandotte hosts.<sup>18</sup> It was soon discovered that pieces from various regions of the blastoderm differed strikingly in their ability to produce pigment in the host. Those which included any part of the head-process, Hensen's node, or a strip 0.3 mm. to each side of them extending from slightly less than 0.1 mm. in front of the process tip to 0.4 mm. behind the primitive pit, produced extensive patches of donor-colored (black) down in the wing and adjacent regions of the Red and the White hosts (Fig. 2). Pieces from all

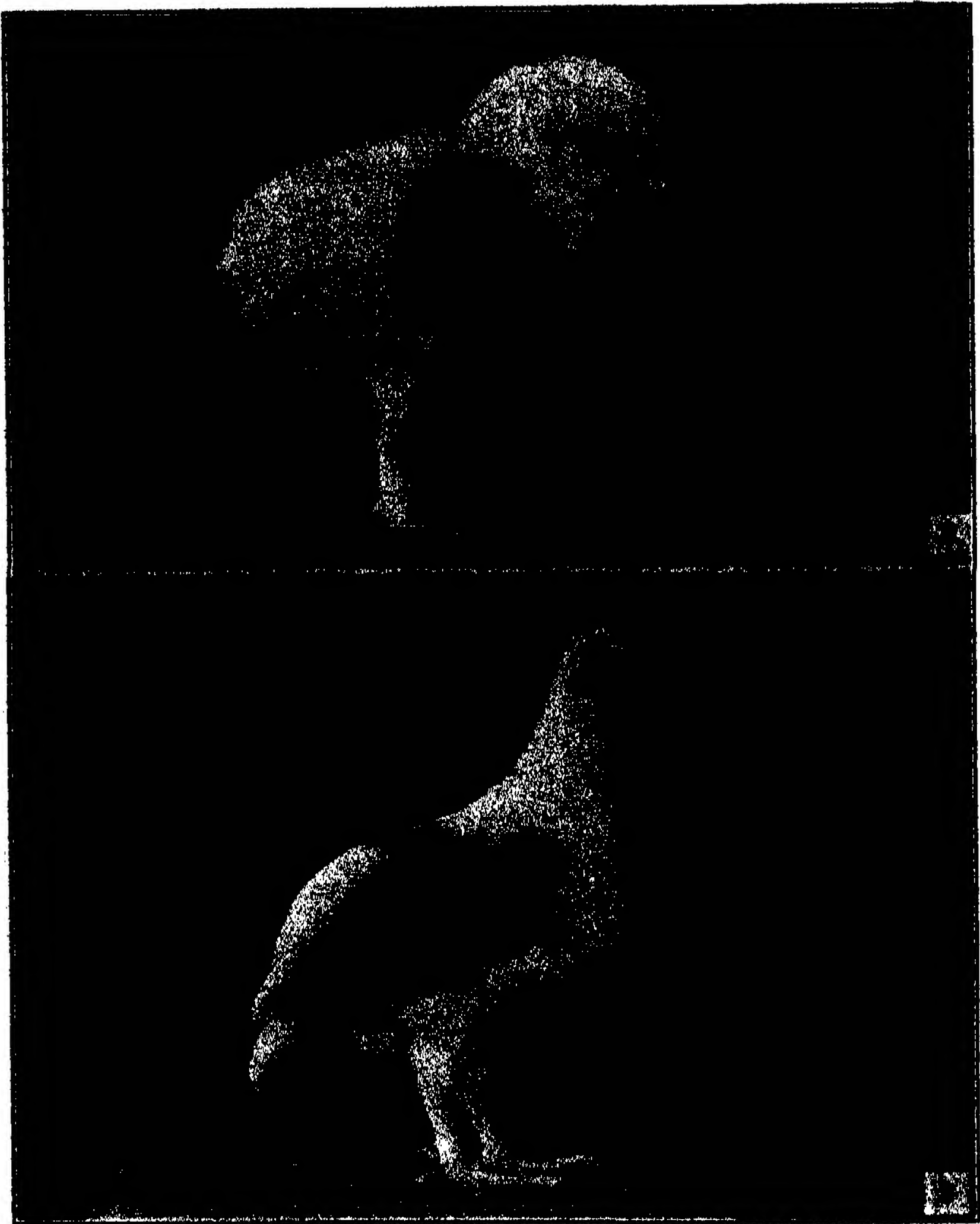


FIGURE 2

White Leghorn chick 3 days after hatching which received in its right wing bud at 75 hours' incubation a transplant including the posterior portion of Hensen's node from a head-process blastoderm (20 hrs.' incubation) of a  $F_1$  hybrid black donor. Note the black (donor-colored) down plumage covering the entire wing.

FIGURE 3

White Leghorn pullet 44 days after hatching, whose right wing bud at 79 hours' incubation received a transplant including a part of the posterior portion of Hensen's node from a definitive primitive streak blastoderm (17 hrs.' incubation) of a Barred Plymouth Rock donor. Note the donor Barred pattern of the juvenile wing plumage.

other regions, i.e., posterior half of the primitive streak, anterior and lateral peripheral portions of the area pellucida invariably gave negative results, i.e., produced no pigment.

In all of these experiments the grafted piece often underwent self-differentiation giving a mass of tissue at the base of the host wing. This was true regardless of whether or not a pigmented area resulted and served particularly well to show that failure to give pigment was no sign that the graft had not persisted.

c. *Primitive streak donors.* Blastoderms showing definitive primitive streaks were tested like the head-process blastoderms just described. Figure 1 shows how the area pellucida is divided into a number of small measured pieces by appropriately placed cuts made with a glass needle. As in the preceding series, so here, it was soon clear that transplants from some portions never produced pigment in the host feathers while those from other parts of the same blastoderm regularly gave extensive donor-colored feathered areas (cf. Figs. 2 and 3). Although the region yielding positive results in the primitive streak blastoderm is quite similar to that of the head-process stages in that it is confined to the region of Hensen's node, it is more restricted in its total antero-posterior length due to the absence of the head-process. Pieces taken outside of an area 0.25 mm. lateral, 0.25 mm. anterior and 0.3 mm. posterior to the primitive pit usually do not produce pigment in the host feathers. In one exceptional case a trace of pigment resulted from grafting a posterior piece cut 0.4 mm. from the primitive pit. The best effect is always brought about by pieces actually containing a part of the node itself.

d. *Unincubated or pre-streak donors.* Barred Plymouth Rock and White Leghorn breeds were used exclusively in this series as donors and hosts, respectively. The donor blastoderm was divided as customary into a number of pieces (9 to 16 usually) somewhat larger than in the preceding series. Cuts were made with reference to the antero-posterior axis which was determined by the somewhat variable rule of von Baer (relation of embryonic axis to egg axis). After the blastoderm was removed from the yolk it was carefully examined under a binocular dissecting microscope for any morphological distinction between anterior and posterior ends. The variability in the anterior extent of the endoderm is often the basis for this distinction. No blastoderm was used which showed any visible evidence of primitive streak formation. In only two cases from a total of 34 which lived past feathering were the host feathers pigmented. In both the entire wing was affected. Since the transplanted piece in these two cases was located within the posterior half of the blastoderm, the region concerned in primitive-streak formation, it would appear that here too the area capable of producing pigment is associated with the primitive streak. Until more positive cases are obtained nothing more precise can be said about its

localization. In ten of the negative cases the grafted piece itself differentiated into a small lump of tissue at the wing base.

The experimental results just described show very clearly that early blastoderms can produce pigment in host feathers long before there is any morphological indication of the neural crest (Figs. 2 and 3). The resultant donor-colored areas thus produced are identical in every respect with those obtained by grafting either neural crest or various tissues of the body containing neural crest cells at later periods in development. We know from these earlier experiments that the feathers of the graft area arise from host epidermal cells;<sup>7,12</sup> the transplant furnishes only the melanophores which migrate into the developing host feather germs where they function perfectly normally in their new environment, even to the extent of reproducing the exact donor feather pattern (e.g., barring, Fig. 3). This effect, however, is not lasting. Ultimately the donor-colored area becomes like the host. This change usually occurs during or at the end of the development of the juvenile plumage, so in relatively few cases donor-colored adult feathers appear. In the present experiments with early blastoderms there has been a marked tendency for the color to persist in the adult feathers of the affected area (wing and breast). In one very nice case obtained from grafting the posterior half of Hensen's node (head-process stage) from a Barred Plymouth Rock donor, the White Leghorn host regenerated a complete set of adult feathers with the Barred pattern. When these were replaced after the first molt the region (wing and adjacent breast) became entirely white (host-colored). So the effect was only prolonged a feather generation. This tendency of the donor-color to persist presents a problem of considerable interest. Perhaps more of the melanophore-forming material is carried into the host with pieces from the node region than with pieces of skin ectoderm or mesoderm of similar size from later stages. Further experimentation is of course necessary to validate this.

*The Relationship of the Pigment-Forming and the Neural-Forming Areas.*—It has been pointed out already that the region of the presomite blastoderm which brings about pigmentation in the host feathers after transplantation is localized about Hensen's node. We know from vital staining experiments that the ectoderm of this region is destined to form medullary plate. Perhaps more relevant here for comparison are the data from numerous transplantation experiments in which the neural-forming potency of measured pieces of the blastoderm of early stages was tested on the chorio-allantoic membrane and *in vitro* cultures.

In the definitive primitive streak stages Hunt<sup>5</sup> found that central nervous tissue occurred regularly in chorio-allantoic grafts of transverse strips of blastoderm taken within 0.2 mm. anterior or posterior to the primitive pit, that is to say, pieces including Hensen's node. While ganglia and nerve fibres appeared occasionally a little further posteriorly (0.28 mm.), no



nervous tissue occurred beyond 0.3 mm. This posterior extent was, however, increased to 0.4 mm. by Rudnick's<sup>9</sup> data from *in vitro* experiments. Although at this stage the lateral extent of nervous tissue development has

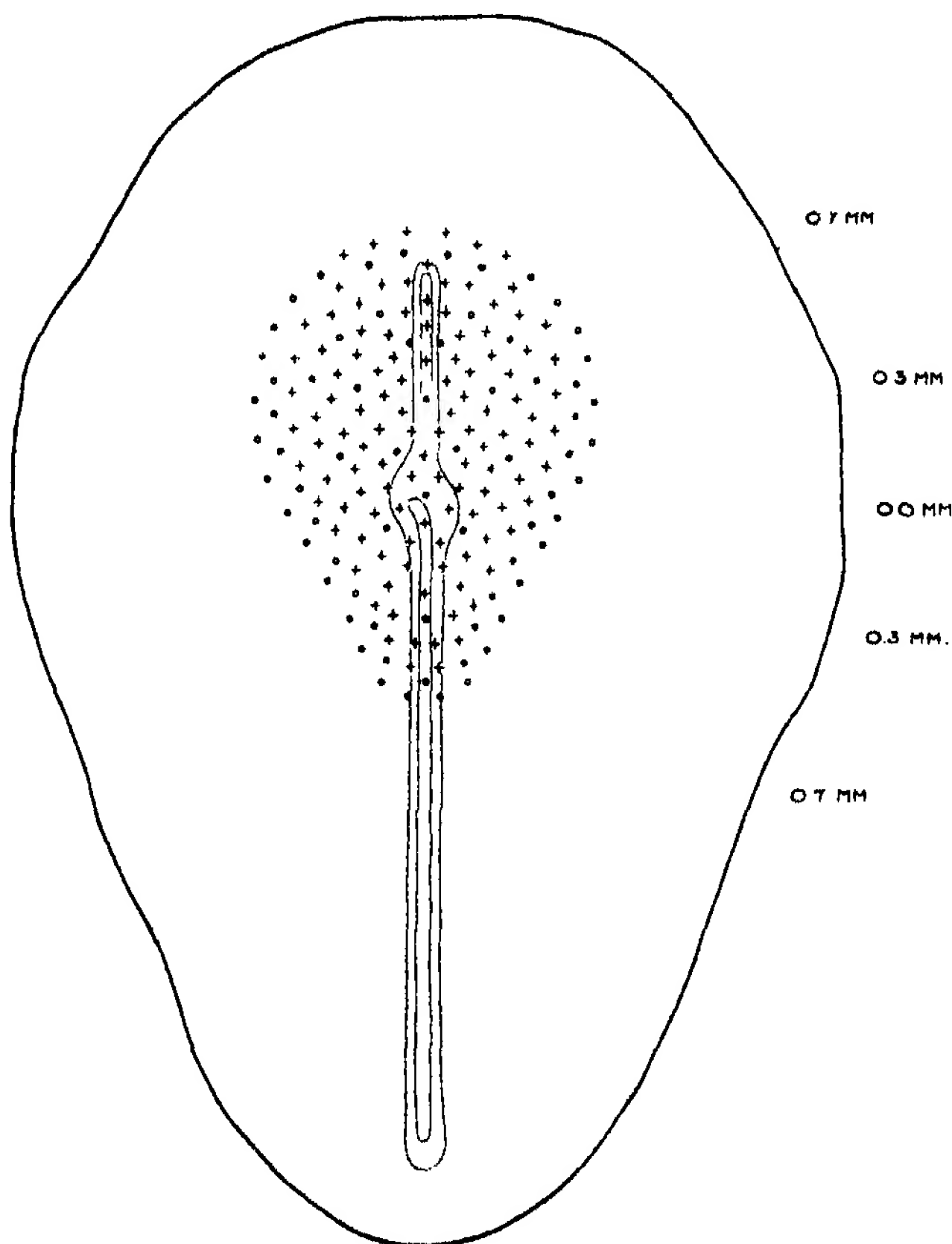


FIGURE 4

Diagram of a chick blastoderm of the head-process stage of development (19-20 hrs.) showing the localization of the area which will produce neural tissue in chorio-allantoic grafts from small measured pieces. (+, brain or neural tube; °, ganglia.) Any part of this same area has the capacity to produce pigment in host feathers.

not been so accurately determined, various data indicate that the neural-forming potency diminishes progressively laterally just as it does anteriorly and posteriorly with increased distance from the primitive pit, falling somewhere between 0.2 and 0.3 mm.

With the development of the head-process (notochord) the antero-posterior extent of the medullary field is increased and the region which will now give nervous tissue in chorio-allantoic grafts is centered about the head-process as well as the node. Figure 4 represents diagrammatically the area from which nervous tissue has developed in chorio-allantoic grafts from small measured pieces taken from blastoderms in the head-process stage.<sup>6</sup> The plus signs indicate central nervous tissue, the circles ganglia. It will be observed that the area capable of developing ganglia is more extensive laterally and posteriorly than that giving brain or spinal cord.

Now if we compare the areas which give rise to pigmented feathers with areas from which nervous tissue developed in chorio-allantoic grafts we see a remarkable agreement in the position and extent of the two in the blastoderm. Any part of this neural-forming area will produce pigment if grafted to a host embryo.

Just as all transplantation experiments point to a progressively diminishing capacity for differentiation of nervous tissue in the lateral and post-nodal portions of the medullary field, so here we find a similar tendency for a diminution in the quantity of pigment produced. As already pointed out, the greatest pigmentation effect was produced by transplants including the node itself or some part of it.

Later on, with the appearance of somites and medullary tube, the node region still continues in its parallel rôle of nervous tissue development in chorio-allantoic grafts and of pigment formation in host feathers. In fact even at 33 and 48 hrs.' incubation (approximately 15 and 30 somites) Waterson<sup>10</sup> has obtained extensive areas of black down feathers on White Leghorn hosts from implants of Barred Plymouth Rock blastoderms containing the node or a part of it.

In the pre-streak (unincubated) stages it has been more difficult to establish a direct connection between the neural-forming and the pigment-forming regions in view of the fact that so few positive cases of pigment formation have so far been obtained. Yet, inasmuch as both of the positive cases came from transplants within the posterior half of the blastoderm, the region definitely concerned with primitive streak formation, they do seem significant and, indeed, sufficient to suggest strongly that the same relationship which has been clearly established for all the other stages examined, does exist here, too. There is certainly no reason on the grounds of embryonic development to expect the unincubated (pre-streak) blastoderm to behave differently in this particular respect. Butler's<sup>1</sup> experiments have shown quite clearly that the posterior quadrant of the blastoderm at this stage has the capacity to develop all the axial embryonic structures with a high degree of differentiation, although with lower frequency than older stages. She found that subdividing the posterior half greatly hindered the expression of its developmental capacity. It is



possible that the very small pieces used in the present experiments may have something to do with the preponderance of negative results. Experiments now in progress should settle this point.

*Evidence of a Progressive Change in the Pigment-Forming Area.*—From the foregoing study we have seen that at the definitive primitive streak stage any part of an area approximately 0.6 mm. long  $\times$  0.5 mm. wide surrounding and including Hensen's node can, if grafted, produce pigment in host feathers. No other region of the blastoderm has this capacity. A little later when the node begins to regress and leave in its wake the differentiated notochord (head-process) and medullary plate we find that the area capable of producing pigment still centers about Hensen's node (0.3 mm. to each side of the primitive pit and 0.4 mm. posterior) but the anterior extent has increased, depending upon the length of the head-process. So, the region now totipotent for pigment formation includes the tip of the process and a region 0.3 mm. to either side (medullary plate). Thus in a medium head-process stage (Fig. 4) the dimensions of this area are approximately 1 mm. long  $\times$  0.6 mm. wide. As development continues anterior to the node with the folding of the medullary plate into a tube and the formation of somites, we find now that any portion of the medullary tube itself will give pigment if grafted but not the adjacent regions as somites, for example. (The node apparently retains its pigment-forming ability as long as it is capable of differentiating nervous tissue.) With the appearance of the neural crest the potency to form pigment becomes restricted to the dorsal half of the neural tube<sup>8</sup> and finally to the neural crest only. Thus we see that in the early periods of development (before the appearance of the neural crest) the potency of the pigment-forming area far exceeds its prospective value. As with other embryonic organ-forming areas such as the eye, heart, kidney, etc., so here, there is a gradual restriction of potency until the formation of a definite primordium. As regards the pigment-forming area, we have seen that in the early developmental stages it coincides with the area which will produce nervous tissue in chorio-allantoic grafts. However, with the appearance of a definite morphological structure—the neural crest—the capacity to form melanin pigment is somehow taken over completely by this particular neural derivative.

<sup>1</sup> Butler, Elizabeth, *Jour. Exp. Zool.*, **70**, 357–389 (1935).

<sup>2</sup> Dorris, Frances, *Ibid.*, **80**, 315–339 (1939).

<sup>3</sup> Eastlick, H. L., *Ibid.*, **82**, 131–150 (1939).

<sup>4</sup> Holmdahl, D. E., *Zeit. mikro-anat. For.*, **14**, 99–298 (1928).

<sup>5</sup> Hunt, T. E., *Anat. Rec.*, **55**, 41–65 (1932).

<sup>6</sup> Rawles, Mary E., *Jour. Exp. Zool.*, **72**, 271–315 (1936).

<sup>7</sup> Rawles, Mary E., *Jour. Gen.*, **38**, 517–532 (1939).

<sup>8</sup> Ris, H. (in press, 1940).

<sup>9</sup> Rudnick, Dorothea, *Jour. Exp. Zool.*, **78**, 369–381 (1938).

<sup>10</sup> Watterson, R. L., *Anat. Rec.*, **72**, Sup. 100–101 (1938).

<sup>11</sup> Willier, B. H., Rawles, Mary E., and Hadorn, E., *Proc. Nat. Acad. Sci.*, **23**, 542-546 (1937).

<sup>12</sup> Willier, B. H., and Rawles, Mary E., *Ibid.*, **24**, 446-452 (1938).

<sup>13</sup> The reciprocal transplantation of White Leghorn to Barred Plymouth Rock and  $F_1$  hybrid black hosts gave negative results just as the earlier experiments with head skin ectoderm.<sup>11,12</sup> While transplants from White Leghorn donors not infrequently produce small areas of white feathers on Black and Buff Minorca hosts, such a result is very rare with Barred Plymouth Rock hosts (4 cases, unpublished data). The behavior of the White Leghorn donors in this respect differs from that of White Wyandottes and White Silkies, which regularly give rather extensive white feathered areas when grafted to pigmented hosts (Barred Plymouth Rock,  $F_1$  hybrid black, Black Minorca).

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*ON THE SYNTHESIS OF CLEAVAGE CHROMOSOMES*

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The question which has prompted the present inquiry is, "How is it possible for the chromosomes in rapidly segmenting eggs to synthesize so quickly the new material needed in the reduplication process prior to each cell division?" While it is not to be supposed that a full answer can be given to this question at present, nevertheless, if we consider modern ideas of protein and chromosome structure and behavior and some other facts scattered in the cytological literature of the past, there emerges a surprisingly simple and illuminating hint as to one of the ways this may be accomplished. Here it is proposed to draw attention to the pertinent evidence and some of its rather obvious or possible implications.

Chemists have shown that proteins are composed of long chain-molecules, each link of the chain being a chemical unit such as an amino-acid residue, a pyrimidine ring or the like, united with other structural units by rather simple bonds. Thus, two amino-acids may react to form a molecule of water and the "residues," as they are called, are linked by a "peptid" bond, and when many units are involved we have a "polypeptid" chain. When new molecules of a complex protein are formed the lattice of the old molecule is supposed to act as a form or mold, each unit of which, such as an amino-acid radical, attracts, or somehow receives from the surrounding medium, a unit like itself and in this way the new molecule is organized and built up. Such a method of formation allows us to understand how the high degree of specificity of proteins (and presumably of genes) is maintained. Viewed in this light, the reduplication 5 to 10 or more times of the same chromosome during 24 hours in segmenting eggs would involve the utilization of relatively large amounts of the constituent units of the nucleoproteins of the chromosomes and would be much more easily understood were we to assume that the egg cytoplasm is extremely rich in the

structural units out of which nucleoproteins are made rather than to assume that these needed materials are synthesized anew, at some phase of the division cycle, out of more or less undifferentiated food material.

The early cytological literature is replete with descriptions of the various cellular mechanisms involved in the formation of yolk within the animal egg. In general there are two common methods, either through the agency of nurse-cells, or by the activity of the ovum itself. The cytological picture of the egg, in these two instances, is very different.

When the eggs grow at the expense of nurse-cells, the latter increase rapidly both in the size of the nucleus and the cytosome, and there is a very marked growth in the amount of chromatin as is shown by Feulgen's nucleal reaction. In the ovary of *Drosophila melanogaster*, for example,<sup>4</sup> the nurse-cell nuclei increase in diameter from 5  $\mu$  to 40  $\mu$  or more. This means that the volume of the original nuclei has been increased about 512 times. Judging from the figures of other investigators of nurse-cells (e.g., Gross,<sup>1</sup> or Jørgensen,<sup>2</sup>) this size increase is in no way unusual. As the growth of the ovum nears completion, the nurse-cell contents are usually absorbed by the egg either through a direct engulfing, as in many Diptera, or more indirectly by absorption through the egg wall, during which process the nurse-cell nucleus loses its chromaticity and dwindles in size along with the cell cytoplasm until the nurse-cell remains are shrivelled bits of débris which completely disappear. Thus directly or indirectly the nurse-cell contents are absorbed and large quantities of nuclear material enter the cytoplasm of the egg. An essentially similar condition obtains in the egg cells of many plants. Generally speaking, when nurse-cells function in yolk formation the egg nucleus remains small and stains very lightly.

When the formation of yolk is carried out by the egg itself, the egg nucleus, or germinal vesicle, invariably shows a great increase in size and this is accompanied by the formation of numerous nucleoli and often elaborate chromatic structures such as the so-called "lampbrush" chromosomes. After the formation of yolk is completed, the amount of chromatin which enters the first polar spindle is only a very small portion of the total amount visible when the germinal vesicle is at the height of its activity. Most of this chromatin, or its derivatives, is discarded into the egg cytoplasm, when the germinal vesicle breaks down, and is absorbed. Thus as regards nucleoproteins the situation is essentially similar to that in eggs nourished by nurse-cells.

In some animals both ovum and nurse-cells may function simultaneously or, as in the case of aphids, the summer eggs may be formed chiefly through the activity of a large germinal vesicle, while the yolk of the slow-growing winter eggs is due to the activity of nurse-cells. In the latter, the egg nucleus remains quite small and relatively achromatic.

The point of central interest for us is that by the cytological mechanisms

employed in the growth of ova prior to fertilization, the cytoplasm of the egg is the recipient of large amounts of chromatin, or its derivatives, either through an engulfing or an indirect absorption of nurse-cells and their nuclei, or from the breakdown of the germinal vesicle.

The facts cited have been known to cytologists for a long time but it is only recently that we have gained a clear insight into the nature of the changes in the chromosomes which go hand in hand with any great increase in nuclear size.

In larval tissues of insects, growth is often accomplished by an increase in nuclear and cell size rather than by cell division. Accompanying this increase in nuclear size Geitler<sup>3</sup> has shown that there is a series of intra-nuclear chromosome divisions so that ultimately these larval somatic cells reach a high degree of polyploidy. For example, the large lobed nuclei in the salivary gland of *Gerris* have either 1024 or 2048 complete sets of chromosomes, oenocytes are 128-ploid and nuclei in the septum walls of the testis are commonly 16-ploid. In Diptera, the salivary gland chromosomes exhibit a special type of polyploidy in which the chromatids (after somatic synapsis) remain closely associated together.

Recently the writer and E. R. Reindorp<sup>4</sup> made a study of the nurse-cells in the ovary of *D. melanogaster* and we found very clear evidence for a series of intra-nuclear chromosome division cycles going hand in hand with an increase in the size of nurse-cell nuclei so that by the time a diameter of 40  $\mu$  is reached there are probably 512 haploid sets of chromosomes present in each nurse-cell. Since there are 15 nurse-cells associated with each egg, in the fruit fly, and these are all eventually absorbed by the egg cytoplasm, it is obvious that prior to fertilization and cleavage the ovum receives the materials of thousands of homologous chromosomes.

No one has as yet made a study of the growth of germinal vesicles to determine if intra-nuclear division cycles occur here, but our modern cytological outlook compels us to infer that the great increase in the chromaticity of these nuclei is due to some sort of reduplication of the constituent chromosomes and there is much evidence already which suggests that "lampbrush" chromosomes may not be simple pachytene chromosomes, but chromosome aggregates. In the first place, if one goes back to Rückert's original description of how lampbrush chromosomes are formed in, for example, *Pristiurus*,<sup>5</sup> we are confronted with the fact that a typical pachytene thread, 10  $\mu$  long and 0.5  $\mu$  in diameter grows into a structure 100  $\mu$  long and 10  $\mu$  in diameter. During this growth Rückert says that the "Mikrosomen" (chromomeres) put out side branches which lie at right angles to the long axis; these side branches are chromomeric in structure and, were one of them to lie separately, it would be considered a single chromosome. These side branches would account for the increase in the breadth of the chromosomes but not their ten-fold increase in length. A



second and very suggestive fact is that in the nurse-cells of *D. melanogaster*, at a definite point in the intra-nuclear division cycle of large nuclei, the homologous chromatids, derived from the repeated divisions of the progeny of a single chromosome, form a hairy-caterpillar-like aggregate which is strikingly similar in form to the lampbrush chromosomes. There is a tendency for the separate chromatids to lie parallel to each other in these aggregates, and were one to transplant such an aggregate to a vertebrate egg undoubtedly it would be called a "lampbrush" chromosome. We are now making at my laboratory a study of the way typical lampbrush chromosomes are formed, but in the meantime it seems reasonably safe to conclude, in the light of all the evidence, that the growth of the germinal vesicle in eggs is accompanied by some sort of reduplication of the constituent chromosomes. As I have pointed out, only a very small part of the chromatin in the germinal vesicle enters the first polar spindle, and the remains of the germinal vesicle are absorbed by the egg plasm. This means that the material from thousands of chromosomes is set free in the cytoplasm, and just as in eggs with the nurse-cell mechanism, this would be available for use by the cleavage chromosomes.

There arises now the question: What happens to the chromosomes (or their derivatives) when they enter the egg cytoplasm, either by the nurse-cell or the germinal vesicle route? That they do not persist as visibly organized structures is well established cytologically and since the most diverse types of eggs have been tested by Feulgen's nucleal stain, with negative results, it appears that nucleic acid, as such, does not persist. On the other hand, there is reason to believe that the constituent proteins and nucleoproteins of the chromosomes do persist in a partially broken down form, because a number of chemical analyses have shown, in diverse eggs, the presence of large amounts of substances closely related to nucleic acid. Thus, in the mature egg of *D. melanogaster* Caspersson<sup>6</sup> has shown by his ultra-violet photo-electric method, that in the egg cytoplasm there is a high concentration of substances containing the pyrimidine ring. And Caspersson and Schultz<sup>7</sup> report that in the eggs of XX females there is appreciably less of this pyrimidine ring bearing material than in XXY eggs. This is in line with what we would expect in view of the fact that this substance is derived from the breakdown of nurse-cell chromosomes. Also in a number of marine and other eggs, many of which are of the germinal vesicle type, Brachet<sup>8</sup> and other workers have found a high concentration of nucleotides which we can understand in view of the large amount of nuclear material which is set free in the egg cytoplasm by the breakdown of the germinal vesicle.

The evidence, then, indicates that in the cytoplasm of all eggs there are the products of thousands of maternal chromosomes. Just in what form the constituent proteins and nucleoproteins exist is a matter for the bio-



chemist to determine. In the meantime, it seems reasonable to conclude that the rapid building up of the cleavage chromosomes is possible in the segmenting egg because the synthesis is more in the nature of a reassembling of already existing materials, such as nucleotides, etc., under the guidance of the active chromosomes, rather than an actual synthesis of the building blocks from relatively simple substances.

The presence of materials derived from a very large number of maternal chromosomes and genes in the cytoplasm of eggs not only allows us to understand the rapid reduplication of the cleavage chromosomes but also gives us a simple explanation for certain types of cytoplasmic or matroclinus inheritance. We commonly think of genes as forming specific substances which react with other cellular constituents to produce, in the end, phenotypic expressions. Furthermore, Ephrussi<sup>9</sup> has shown that substances produced early in ontogeny may persist and affect structures developed in late larval life. This being true we might anticipate that the presence of large amounts of material derived from the maternal chromosomes and genes might sometimes affect the  $F_1$  phenotype irrespective of the genotype of the latter. Many different cases of matroclinus inheritance, especially those which deal with larval characters, seem best understood in this light, and adult characters may occasionally be affected. Thus Noujdin<sup>10</sup> finds that the presence of a  $Y$  chromosome in the female fruit fly tends to suppress mottling and that all the progeny of such  $XXY$  females also show the same suppression no matter what their chromosome and genetic constitution may be. Since all of the eggs of an  $XXY$  female receive from their nurse-cells thousands of  $Y$  chromosomes which enter the egg cytoplasm we may assume that products of these  $Y$ 's persist and either quantitatively or qualitatively function to suppress the mottling. Since, however, the eggs of the  $F_1$  females receive from their nurse-cells the nuclear products of their own genotype, the matroclinus suppression of mottling would persist only through the one generation.

Attention must also be given to the possible influence which the maternal chromosome products in the egg cytoplasm may play in development in general and especially in so-called parthenogenic merogony. The fact that Mrs. E. B. Harvey<sup>11</sup> has succeeded in stimulating enucleated egg fragments to develop in some instances to a morula stage would seem to minimize the importance of the chromosomes and genes in early development. Such a conclusion does not necessarily follow because it is quite possible that in the absence of a normal nuclear set-up, the genes, and possibly the division centers, brought into the egg by the nurse-cell or germinal vesicle route, may function in this type of abortive development.

<sup>1</sup> J. Gross, *Zool. Jahrb. Abt. Anat. Ont.*, 18, 72 (1903).

<sup>2</sup> M. Jørgensen, *Arch. Zellforsch.*, 10, 1 (1913).

<sup>3</sup> L. Geitler, *Chromosoma*, 1, 1 (1939).

- <sup>4</sup> T. S. Painter, and E. R. Reindorp, *Chromosoma*, **1** (1939).  
<sup>5</sup> J. Rückert, *Anat. Anz.*, **7**, 107 (1892).  
<sup>6</sup> T. Caspersson, *Sk. Arch. Physiol.*, sup. to 73 (1936) and unpublished data.  
<sup>7</sup> T. Caspersson, and Jack Schultz, *Nature*, **142**, 294 (1938).  
<sup>8</sup> J. Brachet, *Arch. Biol.*, **48**, 529 (1937).  
<sup>9</sup> B. Ephrussi, *Amer. Nat.*, **72**, 5 (1938).  
<sup>10</sup> N. J. Noujdin, *Nature*, **137**, 319 (1936).  
<sup>11</sup> E. B. Harvey, *Biol. Bull.*, **71**, 101 (1936).

## A NEW INHERITED CHARACTER IN MAN

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Observations on more than 280 human subjects show the existence of two fairly distinct classes with respect to the ability to turn up the lateral edges of the tongue. In typical positive cases the edges can be rolled together over a considerable portion of the distal area of the tongue, while the organ is slightly protruded. In negative cases there is no turning up of the edges at all. A few intermediates have been encountered; and in numerous cases the ability, at first absent, has been acquired by practice. This latter phenomenon is most frequent in children, only one clear case having been found in an adult—and here prolonged efforts were necessary, whereas in children a few hours are sometimes enough. One man reports that he learned the trick as a child, but now has forgotten it and can no longer do it. It should be added that some children, like most negative adults, appear to be unable to learn. In the data that follow, all cases where the ability was at first absent are entered as negative.

Another complication encountered is that a few children are unwilling to show whether they possess the ability or not. In one of these cases the child later said that this unwillingness was due to embarrassment because the ability was absent. The few (two) remaining such individuals have been entered in the tables as negatives.

The ability evidently has no relation to sex, as is shown by table 1:

TABLE 1				
	POSITIVE	NEGATIVE	TOTAL	PER CENT NEGATIVE
Female	88	43	131	32.8 ± 4.1*
Male	95	56	151	37.1 ± 3.9*
Total	183	99	282	35.1 ± 2.8*

\* Standard error.

Studies of families indicate that the ability is inherited, as shown by tables 2 and 3.

TABLE 2

MOTHER	FATHER	NUMBER OF FAMILIES	OFFSPRING		TOTAL
			POSITIVE	NEGATIVE	
Positive	Positive	18	28	5 (1)	33
Positive	Negative	11	16	11 (1)	27
Negative	Positive	14	17	11 (2)	28
Negative	Negative	4	4	9	13
Positive	Unknown	10	14	3	17
Negative	Unknown	2	4	0	4
Unknown	Positive	3	2	2 (1)	4
Unknown	Negative	1	0	1	1

(Numbers in parentheses indicate individuals known to have acquired the ability.)

TABLE 3

NUMBER IN FRATERNITY	NUMBER OF FAMILIES WITH NUMBER OF NEGATIVES INDICATED						
	0	1	2	3	4	5	6
2	11	7	2				
3	3	4	3	1			
4	2	0	0	1	0		
5	2	0	0	0	0	0	
6	0	0	0	1	1	0	0

Table 4 indicates that random mating occurs:

TABLE 4

HUSBAND AND WIFE	NUMBER OF PAIRS	EXPECTED
Positive $\times$ positive	25	27
Positive $\times$ negative	31	28
Negative $\times$ negative	6	7

As will be seen from table 2, neither class breeds true. Positive  $\times$  positive has given five negatives, negative  $\times$  negative has given four positives. The first cases do not seem doubtful, and have come in five separate families. Two of them have each a negative grandparent, and a third has a negative half-brother. This third child learned the trick in one day. The four positives from two negative parents occurred in two families (two in each); both of these families are from fathers with slight speech defects, which suggests that the negative tests on the fathers may be dependent on some additional complicating factor.<sup>1</sup> It is possible, though not proved, that ability to turn up the edges of the tongue may be due to a single dominant gene, with the fairly frequent occurrence of additional complications.

Two pairs of identical twins have been tested. All four individuals (which are included as separate ones in table 3) are positive—a result in

agreement with the supposition of simple inheritance of the character, but needing to be checked by additional observations.

The individuals in table 1 belong to a wide variety of races, but are mostly Americans of mixed European ancestry. Both positives and negatives have been observed in the following groups though the numbers are too small to make the proportions significant: English, Russian, Russian Jewish, Dutch, Polish, Negro (presumably hybrids with whites), Japanese.

The data here recorded have been collected by many observers. Since the members of a given family are usually recorded by the same observer, it might be supposed that the correlation between relatives is a spurious one, due only to differences in classifying intermediates. This supposition is negated by the absence of a correlation between husband and wife, who are also usually recorded by the same observer.

Another possible interpretation of the data is that there is no truly genetic element, the correlations being dependent on family habits or customs, or on imitation in some form. This supposition is not supported by two sets of data: the effect of the father and of the mother on the ability of their offspring is equal; and there is no indication of a striking difference in frequencies of the two classes in the various national and language groups included among those studied.

*Summary.*—The ability to turn up the edges of the tongue, present in about 65 per cent of the persons studied, is conditioned at least in part by heredity.

<sup>1</sup> It should be added, however, that other individuals with slight speech defects have been found to be positive.

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## INFLUENCE OF FEMALE STOCK ON THE FUNCTIONING OF SMALL POLLEN MALE GAMETES

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In the many cases of natural or induced heterozygous small pollen conditions in maize it is now considered axiomatic that the small pollen grains do not function in competition with the normal, large grains. This is true whether the production of small pollen grains is associated with a detectable cytological deficiency or occurs in a stock in which the chromosomes show no visible abnormality.

In the case of small pollen-1,  $sp_1$ , Mangelsdorf<sup>1,2</sup> found that less than one per cent of the  $sp_1$  pollen grains effected fertilization in competition with

normal. He demonstrated, however, that the  $sp_1$  grains are capable of germination and affecting fertilization when screened to eliminate competition with the normal large pollen grains. In our investigations on the small pollen condition we have found, on the whole, that less than one per cent of the  $sp_1$  pollen grains function in competition with normal.<sup>3</sup>

Consequently, it was surprising to find, in 1938, results of some crosses of  $su \times \frac{sp\ su}{+ +}$  that could be explained only by assuming a considerable proportion of the  $sp_1$  pollen grains had accomplished fertilization. The pollinations were made on two different sweet stocks, Purdue 39 and Connecticut 81. Four sets of paired pollinations were made on these two inbreds. In every case the Purdue 39 had a considerably higher percentage of sweet seed indicating that the  $sp_1$  pollen grains were able to function better when applied to this stock. In one case there was 53 per cent of  $su$  kernels. Here there was no competitive effect between the  $+$  and  $sp_1$  pollen grains. The total counts for the pollinations on the two inbreds were as follows:

	$Su$	$su$	TOTAL	PER CENT $Su$ (C. O. CLASS)
Purdue 39 $\times \frac{sp\ su}{+ +}$	2714	1744	4458	39
Conn. 81 $\times \frac{sp\ su}{+ +}$	7089	1478	8567	17

In each of the pollinations on these two inbreds there should have been only about 6 per cent of sweet seeds (the crossover ratio between  $sp$  and  $su$ ) had there been no functioning of  $sp_1$  pollen grains. Both per cents, 17 for C81 and 39 for P39, are much too high for the crossover ratio and indicate a functioning of the  $sp_1$  pollen grains. These figures also show that Purdue 39 silks function as a more favorable host to the small pollen grains than the Connecticut 81, if our interpretation is correct that the higher percentages are due to the functioning of the small pollen grains.

To test this point, the four different classes of seeds from the two pollinations were planted in 1939 and pollen of the plants was examined to determine how many were segregating  $sp_1$ . The following results were obtained:

	POLLEN $sp/+$	POLLEN $+ +$	TOTAL	PER CENT $sp/+$
P39 $\times \frac{sp\ su}{+ +}$ $su$ seed	447	68	515	87
C81 $\times$ " $su$ seed	302	233	535	56
P39 $\times$ " $Su$ seed	9	439	448	2.0
C81 $\times$ " $Su$ seed	18	527	545	3.3

Thus we see the  $Su$  seeds produced a very small per cent of plants with segregating pollen. This was expected since  $Su$  was linked with  $Sp$  and the only segregating plants from these seeds would come from a crossover be-

tween these two loci, and a functioning of the *sp Su* pollen grain after the crossover. Hence the percentage obtained would never be more than the per cent of recombination. The counts are too small to say whether the percentage of 2.0 is significantly different from 3.3.

In the case of the *su* kernels more segregating plants were expected since this is the linkage class of *sp* and *su*. Also a higher percentage was expected from the cross using Purdue 39. The *su* seeds from this cross produced plants, 87 per cent of which had segregating pollen, while the *su* seeds of the C81 cross produced only 56 per cent of segregating plants. These figures verified the assumption that the excess percentages of *su* (C. O. class) seeds in the original cross were really functional small pollen and did not represent an increase in crossing-over. It is possible to obtain the true recombination per cent in each cross by multiplying the original value by the per cent of non-segregating plants found (the true crossovers). When this is done the crossover per cent becomes 5.1 for the P39 crosses and 7.5 for the C81 crosses. These fluctuations from the normal 6 per cent C. O. value are probably not significant.

The pollen examinations showed conclusively there was considerable functioning of *sp<sub>1</sub>* pollen in competition with normal. They also showed a greater functioning when applied to Purdue 39, than when applied to Connecticut 81. The nature of this difference is a matter of speculation. Do the silks of Purdue 39 afford a better environment for the germination of *sp<sub>1</sub>* pollen grains? If so, is it possible by using this inbred as a female stock to secure the functioning of other small pollen male gametes? Other inbreds might conceivably be as favorable or more favorable than Purdue 39.

No explanation of this condition is available at present.

<sup>1</sup> Mangelsdorf, P. C., *Proc. Nat. Acad. Sci.*, 17, 698-700 (1931).

<sup>2</sup> Mangelsdorf, P. C., *Jour. Hered.*, 23, 289-295 (1932).

<sup>3</sup> Singleton, W. R., *Proc. 6th Internat. Genetics Congress*, 2, 182-184 (1931).



*GALACTIC AND EXTRAGALACTIC STUDIES, V. THE PERIOD  
FREQUENCY OF CLASSICAL CEPHEIDS IN THE MAGELLANIC  
CLOUDS*

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Read before the Academy, October 24, 1939

In the study of the characteristics of stellar variation, a well-known observational result is the scarcity of periods for classical Cepheids<sup>1</sup> in the interval from 1.0 to 2.5 days and the conspicuous maximum in the period-frequency curve between four and five days. The intrinsic luminosity, the mass, mean density, temperature, diameter, color, spectrum, and all variations of the five-day Cepheid are naturally accepted as the typical characteristics for theoretical studies. There has been some question as to the reality of secondary maxima in the period-frequency curve at ten and sixteen days; but no doubt has arisen about the principal maximum, which as shown in figure 1 occurs at about 4.75 days, with the curve falling steeply on both sides, approaching minima near two and nine days.

In the present communication it will be shown that in the Small Magellanic Cloud, where we are much more free from the evils of selection than we are in the galactic system, the maximum is in the vicinity of two days. The long-accepted "infrequency" between 1.5 and 2.5 days has disappeared. It is probable that our sampling of classical Cepheids around the sun, although it involves more than three hundred stars, has been insufficient to yield full information on this useful characteristic of Cepheid variation—the distribution of periods; or, alternatively, we might conclude that, improbable as it seems, the galactic system and the Magellanic Clouds differ fundamentally in this one respect—the period distribution of their Cepheids.

Since the new study of the Magellanic Clouds also shows that the distribution of periods depends significantly on the distribution of stellar mass, it might be more correct to say that in the Small Magellanic Cloud as a whole the period frequency of classical Cepheids differs from that in our part of the galactic system either because of a different average star density or because selection has given an untrue picture of the phenomenon for galactic Cepheids.

1. *Period Frequency in the Galactic System.*—From the *Vierteljahrschrift* Catalogue for 1939, the distribution of the periods of the 288 classical Cepheids with periods less than twenty days is given in table 1 and shown as a frequency graph in figure 1, where the plotted dots indicate numbers for each half-day interval and the open circles represent running means of three. There are nearly forty Cepheids now known with periods greater

than twenty days, but the distribution of their periods is here ignored as irrelevant. The minima in the period-frequency curve at 1.5 and at 9.0 days, noted by the Gaposchkins<sup>2</sup> and others, are shown in this plot. Most of the stars with periods less than 2.5 days are discoveries of recent years when the surveys have gone to fainter stars in Milky Way fields.

2. *First Comparison with the Small Magellanic Cloud.*—The period frequency of galactic Cepheids may be next compared with the published results for the Small Magellanic Cloud from which a somewhat comparable frequency curve is obtained. Omitting the periods of 22 outlying Cepheids, published in *Harvard Annals*, 90, No. 4 (1934) and *Harvard Circular* 374 (1932), we obtain the results as given in the first line of table 2 and in fig-

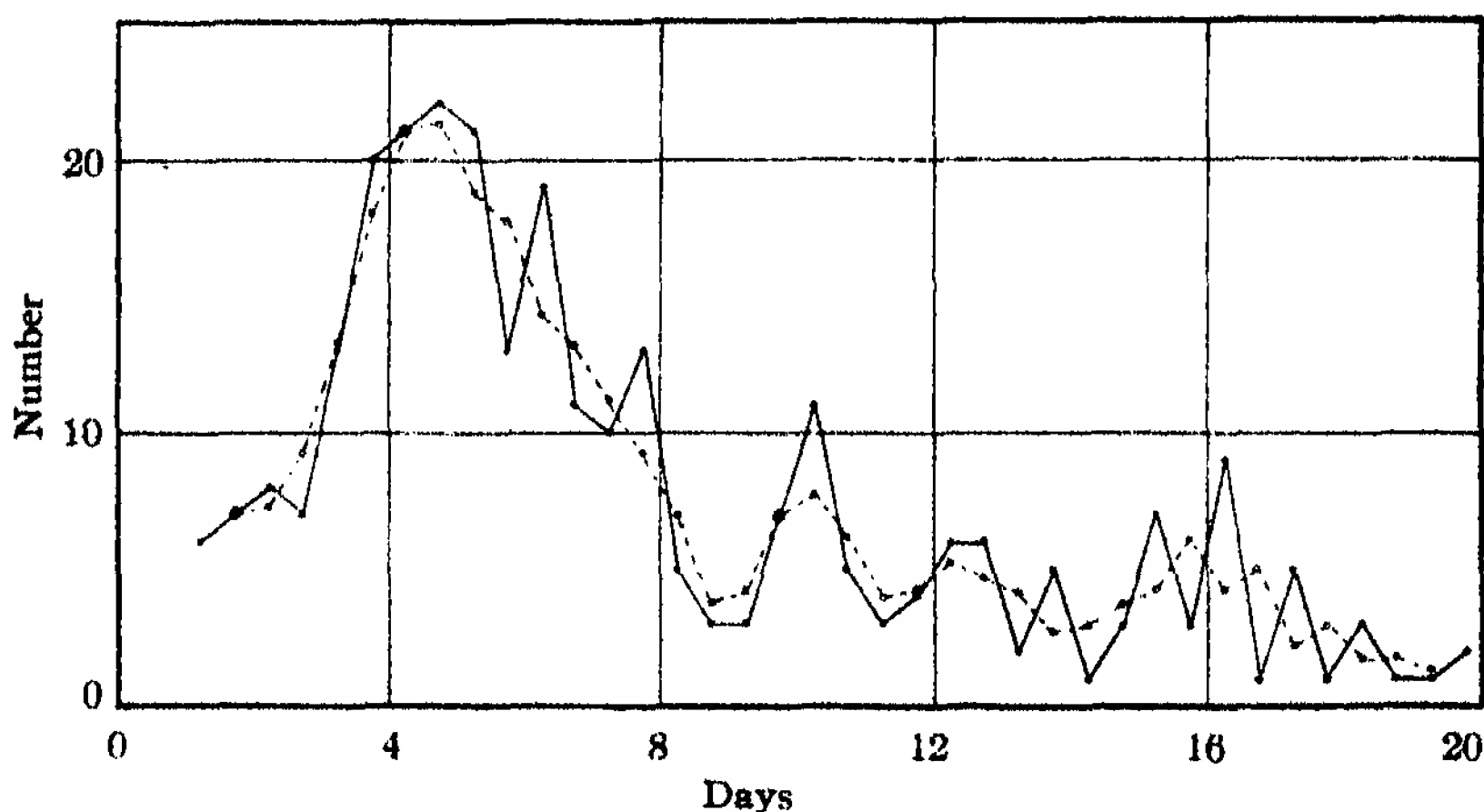


FIGURE 1

Distribution of periods for 288 galactic Cepheids. Ordinates are numbers of variables in half-day intervals of the period (abscissae). Broken lines with open circles represent running means of three points.

ure 2. The maximum of this frequency curve also comes at 4.75 days, or when smoothed at 4.25 days. It is noted, however, that there are here relatively more of the shorter periods, and when we examine the results in *Harvard Circular* 374, where there are many periods less than three days, considerable doubt arises as to the similarity of the frequencies in the Small Cloud and the galactic system.

3. *A New Investigation of the Small Cloud Variables.*—In order to examine the phenomenon further, additional series of plates have been obtained with the Bruce refractor at the Boyden Station. During the past fifteen months approximately 14,000 observations have been made; a sixth of the known variables are now measured. Many of the periods and light curves previously published have been re-examined. With one excep-

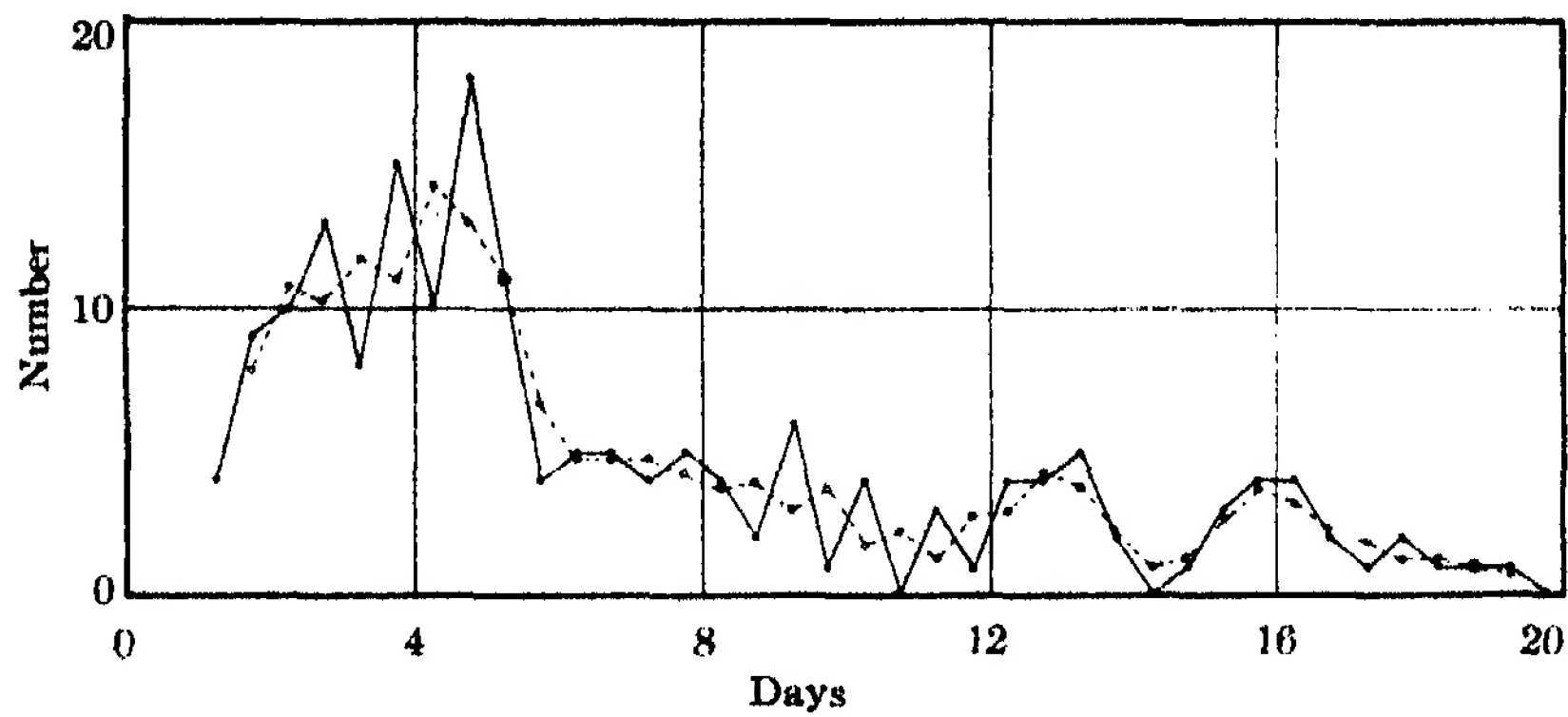


FIGURE 2

Distribution of periods in main body of Small Magellanic Cloud. Coördinates as in figure 1.

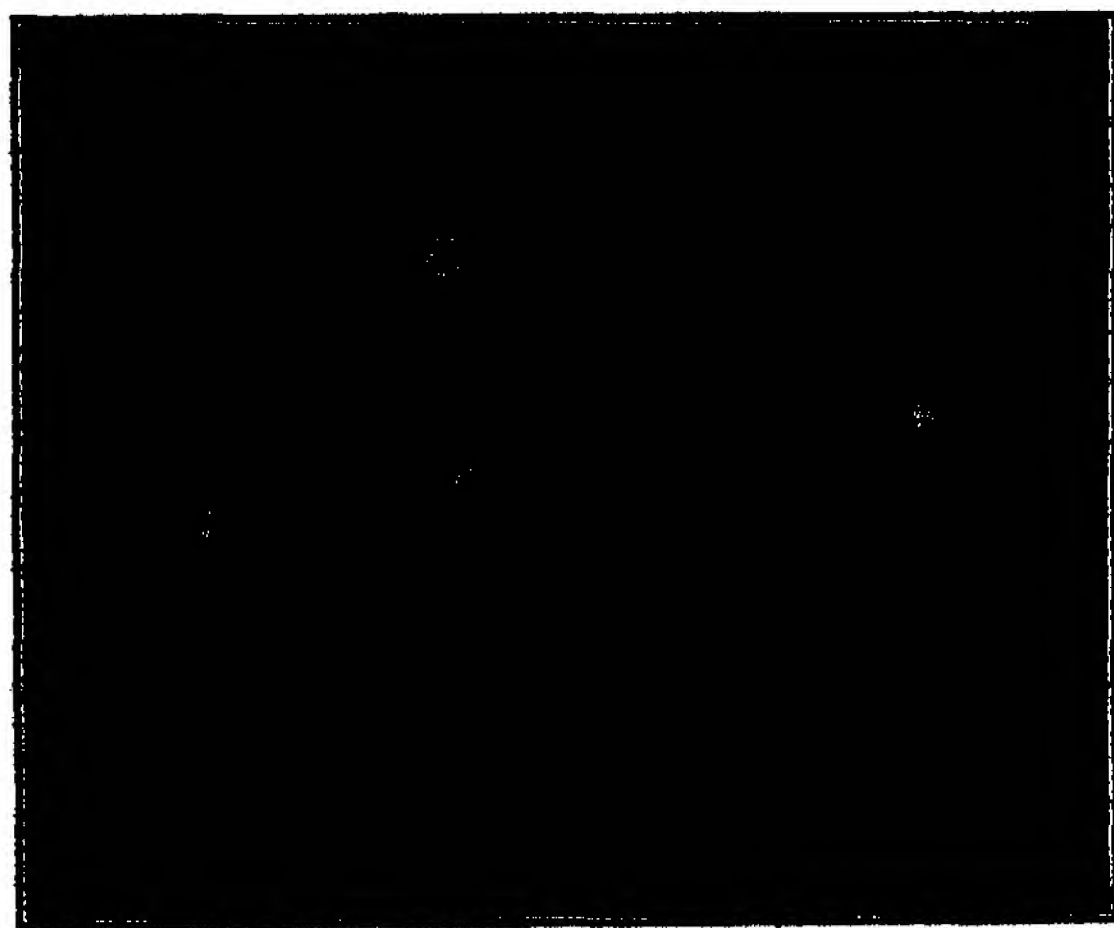


FIGURE 3

The Small Magellanic Cloud, with special regions marked (see table 2 and figures 4 and 5).

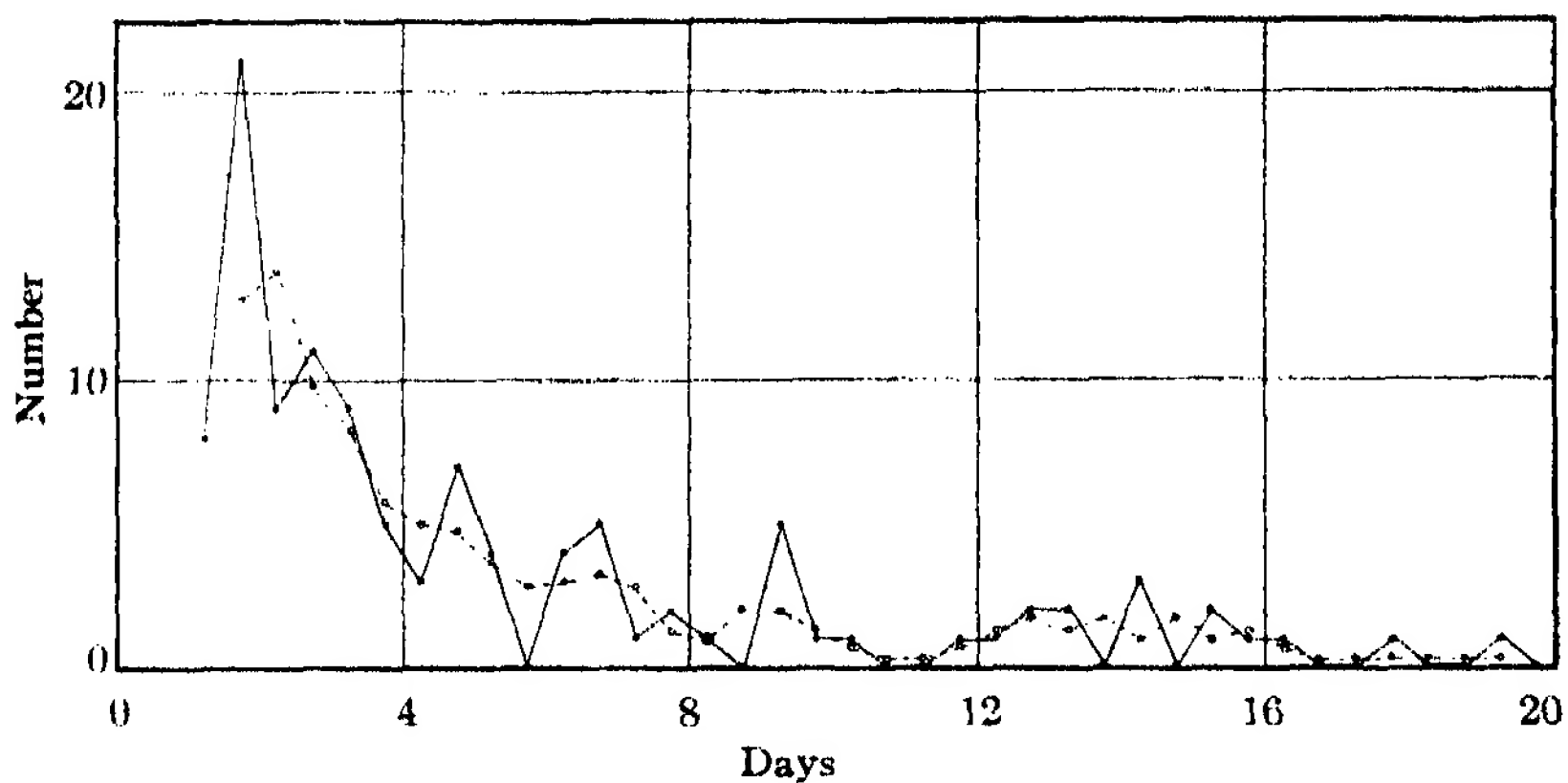


FIGURE 4

Distribution of periods for the fully investigated regions of the Small Magellanic Cloud. Coördinates as in figure 1.

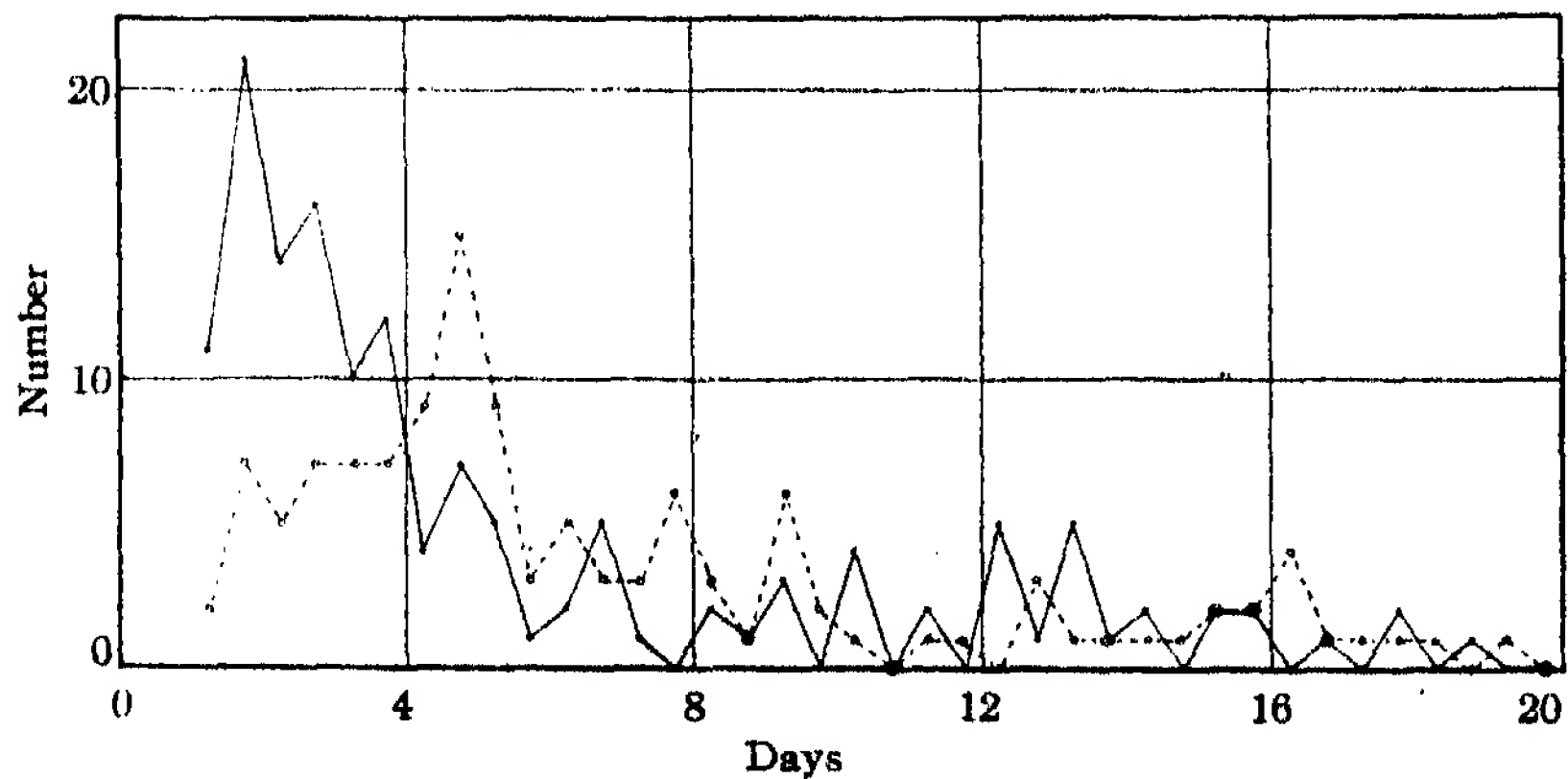


FIGURE 5

Distribution of periods in the "inner" and "outer" regions of the Small Magellanic Cloud, the broken line with circles representing the former. Coördinates as in figure 1.

tion, H. V. 216, the periods obtained by Miss Sawyer for the region near the globular cluster NGC 362 at the northern edge of the Cloud are verified.<sup>8</sup> This northern area has been enlarged and the Cepheids measured in that region more than doubled in number. New investigations in other parts of the Cloud were also undertaken when it became clear that not only should the period-frequency relation be revised but that the possible dependence of period length on star density must also be examined. The eighty new periods determined in the course of this work will be published elsewhere.

In order to examine in detail the distribution of periods throughout the Cloud, we have made table 2, in which the first column indicates various regions in the Cloud, and the tabulated quantities are the numbers of variables in half-day intervals of period, as indicated at the top of successive columns. The regions are marked also on the photograph reproduced in

TABLE 1  
FREQUENCY OF PERIODS FOR GALACTIC CEPHEIDS (1 TO 20 DAYS)

PERIOD IN DAYS	NUMBER	PERIOD IN DAYS	NUMBER	PERIOD IN DAYS	NUMBER	PERIOD IN DAYS	NUMBER
1.0-1.5	6	6.0-6.5	19	11.0-11.5	3	16.0-16.5	9
1.5-2.0	7	6.5-7.0	11	11.5-12.0	4	16.5-17.0	1
2.0-2.5	8	7.0-7.5	10	12.0-12.5	6	17.0-17.5	5
2.5-3.0	7	7.5-8.0	13	12.5-13.0	6	17.5-18.0	1
3.0-3.5	13	8.0-8.5	5	13.0-13.5	2	18.0-18.5	3
3.5-4.0	20	8.5-9.0	3	13.5-14.0	5	18.5-19.0	1
4.0-4.5	21	9.0-9.5	3	14.0-14.5	1	19.0-19.5	1
4.5-5.0	22	9.5-10.0	7	14.5-15.0	3	19.5-20.0	2
5.0-5.5	21	10.0-10.5	11	15.0-15.5	7		
5.5-6.0	13	10.5-11.0	5	15.5-16.0	3		

figure 3. The total of 295 accepted classical Cepheid variables in the Small Cloud for which periods are now determined includes 72 for which periods are longer than twelve days. Since the distribution of these longer periods has no effect on the question of the most frequent period and the location of maxima and minima in the part of the frequency graph in which we are now interested, they have not been used in determining the median period, given for the various parts of the Cloud in the last column of table 2. But the accompanying figures include the data on periods up to twenty days, and for only thirty stars does the period exceed that value.

It should be emphasized that some of the data of table 2 are affected by selection. Until the present investigation was undertaken, the Cepheids chosen for period determination were generally those that could contribute effectively in the testing of the form of the period-luminosity relation, or those that for one reason or another were easiest to measure. As a result there has been a definite selection of the brighter stars and therefore of the

TABLE 2  
DISTRIBUTION OF PERIODS IN THE SMALL MAGELLANIC CLOUD

		REGION IN CLOUD	PERIOD INTERVAL (IN DAYS)															TOTAL NUMBER	MEDIAN PERIOD $\bar{d}$
			1.0-1.5	1.5-2.0	2.0-2.5	2.5-3.0	3.0-3.5	3.5-4.0	4.0-4.5	4.5-5.0	5.0-5.5	5.5-6.0	6.0-6.5	6.5-7.0	7.0-7.5				
		All Published*	4	9	10	13	8	15	10	18	11	4	5	5	4				
A.		Northern Border	5	11	2	7	5	2	1	2	2	..	2	1	..				
B.		Southeast Border	1	6	5	1	1	2	..	1	1	..	..	2	..				
		Both Borders	6	17	7	8	6	4	1	3	3	..	2	3	..				
C.		Nuclear	..	2	3	2	2	5	3	8	6	2	3	2	3				
		(a) Core	..	..	..	1	..	1	..	2	1	..	1	1	1				
D.		Intermediate	2	4	2	2	3	..	2	2	..	..	1	1	..				
		(a) Dense	1	1	..	1	1	..	2	2	..	..	1	..	..				
		(b) Sparse	1	3	2	1	2	..	..	..	..	..	1	..	..				
E.		Inner	2	7	5	7	7	7	9	15	9	3	5	3	3				
		Outer	10	21	14	16	10	12	4	7	5	1	2	5	1				
		Total	12	28	19	23	17	19	13	22	14	4	7	8	4				
		REGION IN CLOUD	7.5-8.0	8.0-8.5	8.5-9.0	9.0-9.5	9.5-10.0	10.0-10.5	10.5-11.0	11.0-11.5	11.5-12.0	>12							
		All Published*	5	4	2	6	1	4	..	3	1	61	203	4.50					
A.		Northern Border	..	..	..	1	..	..	..	..	..	1	42	2.77					
B.		Southeast Border	..	1	..	1	..	1	..	..	..	13	36	2.48					
		Both Borders	..	1	..	2	..	1	..	..	..	14	78	2.70					
C.		Nuclear	2	3	1	4	1	..	..	1	..	20	73	5.12					
		(a) Core	..	..	..	2	1	..	..	..	..	10	21	6.47					
D.		Intermediate	2	..	..	1	..	..	..	..	1	2	25	3.17					
		(a) Dense	1	..	..	1	..	..	..	..	1	1	13	4.70					
		(b) Sparse	1	..	..	..	..	..	..	..	..	1	12	2.16					
E.		Inner	6	3	1	6	2	1	..	1	1	35	138	4.85					
		Outer	..	2	1	3	..	4	..	2	..	37	157	2.99					
		Total	6	5	2	9	2	5	..	3	1	72	295	3.75					

\* Except those in *Harv. Circ.* 374, and *Harv. Ann.*, 90, No. 4.



longer periods. Only in regions *A*, *B*, *D* and the "core" of *C* have all the variables been investigated, whatever the brightness and period.

The deliberate preference shown brighter stars in the determination of periods for the 177 variables represented in figure 2 has tended to force the resemblance of the Cloud's frequency curve to that of the galactic system (Fig. 1). If we make a frequency graph for only the four regions essentially free of selection, we obtain the result shown by figure 4—a surprisingly different picture than heretofore seen for the distribution of Cepheid periods. This might be taken as the true period distribution for Cepheids,

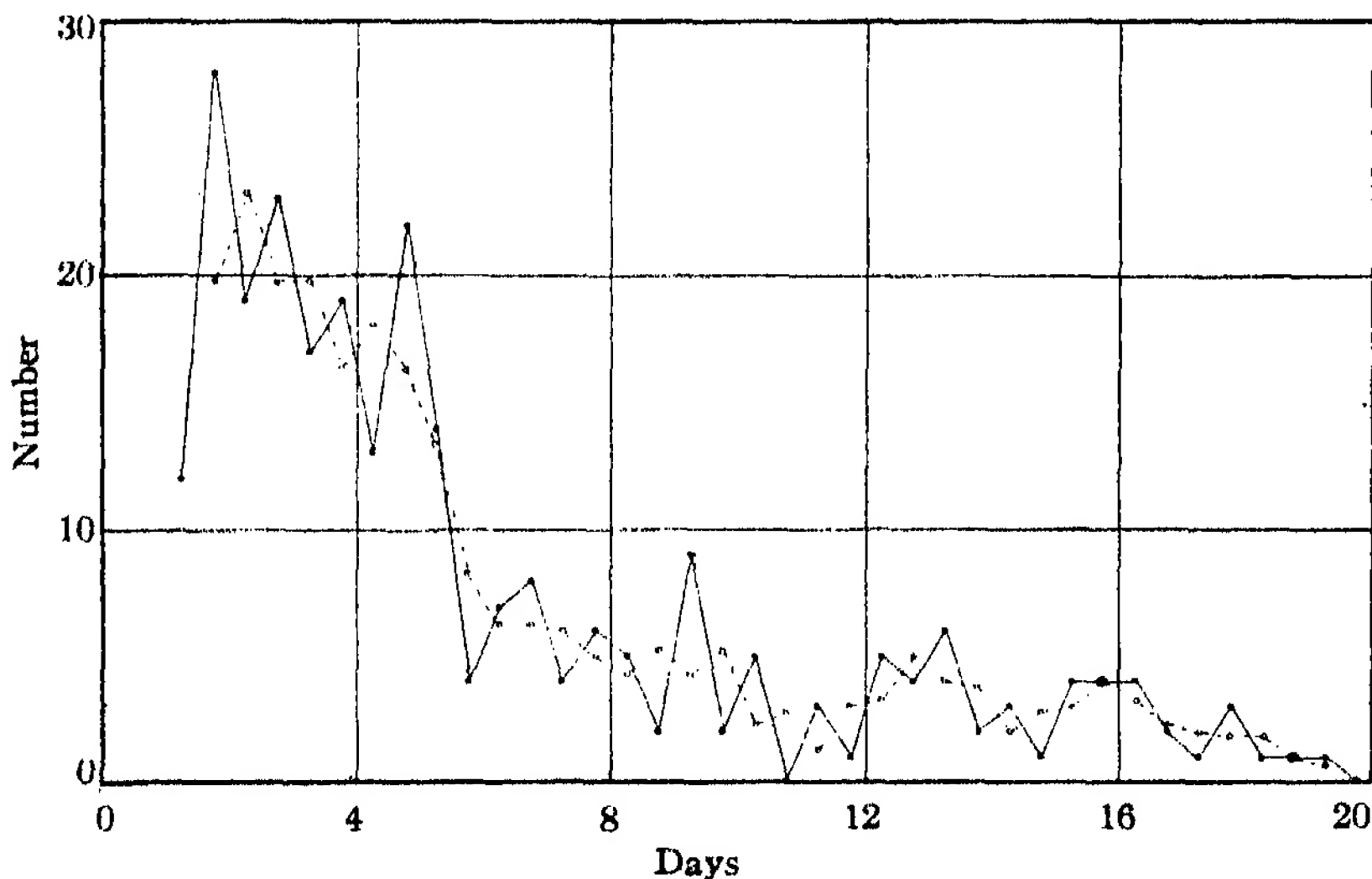


FIGURE 6

Distribution of the 265 periods now known for the Small Magellanic Cloud—the sum of the two curves in figure 5. The broken line with open circles is the running-mean curve. A remnant of the selection effect is seen in the secondary maximum at 4.75 days. Coördinates as in figure 1.

but the four regions involved lie mostly in areas of low star density and may not thoroughly represent the Cepheid population of the Cloud as a whole.

That the period frequency may depend sharply on the star density is shown, without much weight because of the small numbers involved, by the distribution of periods in region *D*—a small area chosen to cut across the border between high and low densities. When we divide this "intermediate" region into equal parts, as in table 2 and figure 3, we find that three-fourths of the periods in the sparse half are less than 3.5 days; nearly three-fourths of those in the denser half are greater than 3.5 days. The medians are 2.16 and 4.70 days, respectively.

Only a small section of the so-called nuclear region, *C*, was thoroughly investigated for the whole range of magnitude and period. In that "core" the median for stars with periods less than twelve days is 6.47 days, a value that may hold for the whole of the nucleus when all of its fainter classical Cepheids have been studied. It is noteworthy that about half the stars of the core have periods greater than twelve days, a ratio not approached in any other area studied. The considerable number of long-period Cepheids in region *B*, however, demands further investigation; these variables may be related to a peculiar extension of the Small Cloud toward the southeast, which we are now investigating as to population and structure.

The three bottom lines of table 2 are shown graphically in figures 5 and 6. For figure 5 all the 295 variables with known periods are divided into "inner" and "outer," in order to show with greater weight and clarity the dependence of the period distribution on star density. If the selection, mentioned above, were absent throughout the areas studied, the maxima of both curves would probably be shifted somewhat toward shorter periods.

The combination of the two curves of figure 5 is shown in figure 6, with the open circles again indicating running means of three. The maximum frequency in this "total" curve is at two days, instead of at 4.75 days as found for galactic Cepheids in figure 1 and for Magellanic Cepheids in the earlier study represented by figure 2. The minimum at nine days has now disappeared. The waves in the frequency curve for periods greater than ten days are perhaps not significant.

Probably it is too early in the study of period distribution to attempt the interpretation of the frequencies observed in the Small Cloud. It is not likely that any other significant selection effects are involved; and the plate material is now quite sufficient to provide ample tests of periods, magnitude sequences and background effects. We might suggest that equipartition of energy may account for the fact that the shorter period variables, with their lower luminosities and masses, appear preferentially in the regions of lower star density; but a simple calculation shows how relatively little the masses of two-day Cepheids differ from those of six-day period. Such fine sorting out on the basis of mass would probably demand an unacceptably long time scale and should require also other clear evidences of an approach toward a steady state.

An alternative hypothesis is that the distribution of matter and gravitational potential throughout the Cloud at some earlier time, or the distribution of the various chemical elements,<sup>4</sup> governed the masses and therefore the periods of the forming Cepheids; in other words, heavy stars formed in "heavy regions," or the chemical elements that favor the maintenance of long-period Cepheid variation may have been localized in the denser regions.

4. *Cepheids in the Large Cloud.*—It will be useful for the interpretation

of the characteristics of the period-frequency graphs in the Small Cloud to see what evidence there is elsewhere for a star-density effect on period distribution. Table 3, similar to table 2, shows what we can now say about the Large Magellanic Cloud. The variables already published<sup>1</sup> show the usual value of the median period, about 4.5 days. Selection has again entered through our special interest in improving the brighter end of the period-luminosity curve. For an area on the axis of the Cloud, and for another of lower star density which is, however, not so near the border as was attained for the Small Cloud, we have made a thorough study and find median periods of 5.24 and 4.36 days, respectively. The effect is in the same direction as for the Small Cloud, but no periods less than 2.5 days have as yet been found. On four special series of Bruce plates, which permit a better border patrol, the question will be further investigated. Mr. A. L. Jennings and Miss Frances W. Wright have assisted in the study of the Large Cloud.

5. *Further Consideration of Galactic Cepheids.*—We have carried through many studies of the bearing of location in the galactic system on the period frequency of classical Cepheids, with the following principal results:

a. The material for galactic Cepheids has been highly selected on the basis of apparent magnitude and convenience of the ob-

TABLE 3

## DISTRIBUTION OF PERIODS IN THE LARGE MAGELLANIC CLOUD

REGION IN CLOUD	PERIOD INTERVAL (IN DAYS)													TOTAL NUMBER	MEDIAN PERIOD $\bar{p}$
	1.0-1.5	1.5-2.0	2.0-2.5	2.5-3.0	3.0-3.5	3.5-4.0	4.0-4.5	4.5-5.0	5.0-5.5	5.5-6.0	6.0-6.5	6.5-7.0	7.0-7.5		
All Published*	..	..	..	8	8	12	10	10	2	2	3	5	3		
Axis	..	..	..	..	3	2	..	2	2	2	..	2	..		
Outer	..	..	..	1	2	3	2	2	1	..	..	1	..		
Total	..	..	..	9	13	17	12	13	5	4	3	8	3		
REGION IN CLOUD	7.5-8.0	8.0-8.5	8.5-9.0	9.0-9.5	9.5-10.0	10.0-10.5	10.5-11.0	11.0-11.5	11.5-12.0	>12					
All Published*	1	2	..	1	1	1	1	2	1	33	106				
Axis	1	1	1	..	..	..	..	..	..	2	18				
Outer	1	..	..	..	1	..	..	..	1	..	15				
Total	2	3	1	1	2	1	1	2	2	35	137				

\* This number includes three unpublished periods by Miss Sawyer and six by Miss McKibben.

server (latitude, seasons, equipment). Also the irregular distribution of space absorption and the irregular structure of surrounding star fields combine to make valid conclusions impossible.

b. The periods average shorter in higher latitudes, where the star density is certainly lower;

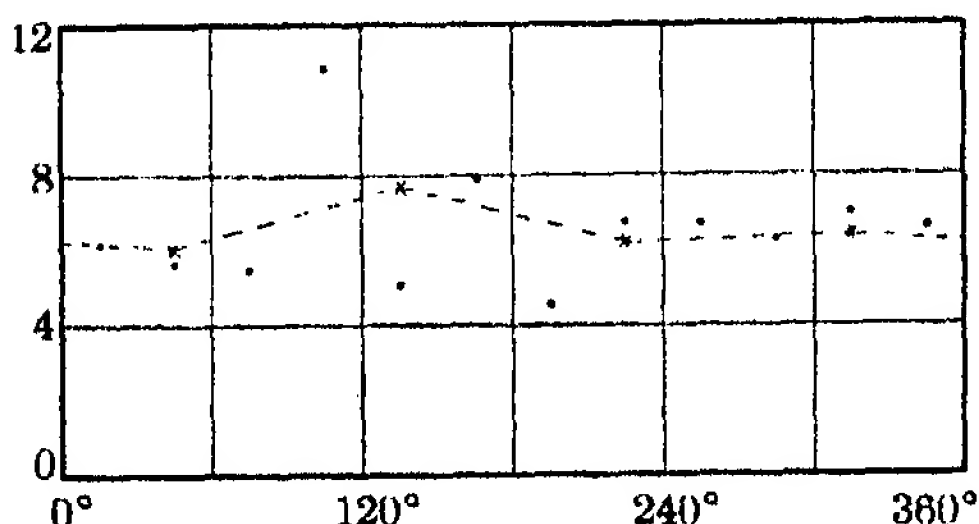


FIGURE 7

The relation of median period (ordinates) to galactic longitude (abscissae) for galactic Cepheid variables with periods less than twenty days. The crosses represent median periods for  $90^\circ$  intervals of longitude; the dots for  $30^\circ$  intervals.

all galactic Cepheids with median apparent magnitudes brighter than 10.0 and periods less than twenty days:

$\lambda$	$15^\circ$	$45^\circ$	$75^\circ$	$105^\circ$	$135^\circ$	$165^\circ$	$195^\circ$	$225^\circ$	$255^\circ$	$285^\circ$	$315^\circ$	$345^\circ$
$P$	6.1	6.6	5.4	10.8	5.0	7.9	4.5	6.7	6.7	6.3	7.0	6.6
No.	9	10	9	6	5	7	6	9	23	12	5	9

The Cepheids in the direction of the galactic center (longitude  $325^\circ$ ) may on the average lie in regions of greater star density and mass than prevail in the anticenter direction, but we cannot be certain because of our present ignorance of the details of galactic structure.

d. It is easy to show that our records for galactic Cepheids are progressively less complete for the more distant stars, those of shorter period being preferentially omitted so long as we do not approach the boundaries too closely.<sup>7</sup> We conclude, therefore, that for a given volume of space in the galactic system the period-frequency maximum would shift toward shorter periods than suggested by figure 1. No matter how complete the survey, however, we may not be able to attain in this part of the galactic system results comparable to those for the Small Magellanic Cloud (figure 4 or 6), since it is possible that the star density near the Sun corresponds not to the average but to the densest part of the Small Cloud.

*Summary.* a. The periods and median magnitudes of eighty classical Cepheid variables in the Small Magellanic Cloud have been determined in

density is certainly lower; but since the boundaries of the galactic system enter the problem, another kind of selection may be largely responsible for this result also.

c. Figure 7 shows that there is no appreciable dependence of period-length on galactic longitude in the material now available.<sup>6</sup> It is based on the following median values of galactic longitude and period (number of stars in last line) for

order to supplement earlier material for an investigation of the form of the period-frequency curve and the dependence of median period on position in the star cloud.

b. It is found that the maximum frequency at about 4.5 days for galactic Cepheids holds also in the Small and Large Clouds when a strong selection for the bright long-period Cepheids affects the material, but when the selection is eliminated the maximum of the frequency curve shifts to approximately two days, where heretofore a minimum of frequency had been supposed to occur.

c. Between the center and the borders of the Small Cloud the median frequency differs by two or three days (table 2), an indication that the Cepheids in the regions rich in stars are systematically brighter and more massive than in the outer parts of the Cloud.

d. Preliminary results for the Large Magellanic Cloud are presented. An investigation of the galactic Cepheids with respect to the dependence of period on position in the Milky Way gives inconclusive results, probably because of the many vitiating factors of selection in the discovery and analysis of galactic variable stars.

<sup>1</sup> "Classical" Cepheids are those with periods in excess of one day. For recent discussions of period distribution see, for example, Lundmark, K., *Vierteljahrsschrift d. Ast. Gesell.*, **68**, 377 ff. (1933); Campbell, L., *Pop. Ast.*, **47**, 216 (1939).

<sup>2</sup> Gaposchkin, C. P., and S., *Variable Stars*, Harvard Observatory Monograph No. 5, 162 (1938).

<sup>3</sup> But the uncertainties in range and period for H. V. 1869 lead us to omit it for the present.

<sup>4</sup> The possibility that the abundance of lithium, beryllium, boron and deuterium may have a bearing on the form of the period-frequency curve of galactic Cepheids has been suggested by Gamow, *Nature*, **144**, 575 (1939).

<sup>5</sup> *Harv. Bull.* 905, 22 (1937).

<sup>6</sup> With less material the relation of period to longitude has been previously examined by Lundmark (see footnote 1) and earlier by Schilt, *Ap. J.*, **64**, 149 (1926), and *Ast. Jour.*, **38**, 197 (1928). In our tabulation and plot we omit variables with magnitudes fainter than 10.0 because of the proximity of the boundary of the galactic system in the anti-center direction and the consequent restriction on average period length—high luminosity stars in those longitudes being necessarily absent among Cepheids of faint apparent magnitude.

<sup>7</sup> Some of the effects of selection will be discussed and evaluated in a paper appearing elsewhere.



*REMARKS ON ZWICKY'S PAPER "ON THE FORMATION OF CLUSTERS OF NEBULAE AND THE COSMOLOGICAL TIME SCALE"*

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In a paper recently published in these PROCEEDINGS<sup>1</sup> Zwicky has marshalled arguments purporting to show that the existence of large clusters of nebulae is inconsistent with the cosmological short time scale demanded by the theory of the expanding universe. His main argument appears to be that the formation of clusters of nebulae requires multiple close encounters; an estimate of the interval between collisions leads him then to the conclusion that the time of formation of a large cluster out of a random distribution would be greater than  $10^{18}$  years, a figure obviously so much greater than the age of expanding universe as to rule out the latter. He then examines the possibility that clusters might already have existed in the early stages of the expansion and dismisses it on the ground that, according to the investigations of Sinclair Smith, the velocity distribution in the central part of a cluster is the same as at a considerable distance from the center, and large radial velocities at the rim of a cluster are not observed, as would be expected if they had shared in the expansion of the universe. He finally suggests an experimental verification of the theory of the expanding universe which would consist in attempting to find out if remote clusters are more condensed than nearby ones.

While formulating these objections to the theory in question, Zwicky seems to have overlooked certain important results of Lemaitre on the theory of the formation of clusters of nebulae in an expanding universe. In a paper appearing almost six years ago in these PROCEEDINGS<sup>2</sup> he showed that in such a universe there may be locally collapsing regions and equilibrium regions, and went on to examine the hypothesis that the former are to be identified with the extragalactic nebulae and the latter with the clusters of nebulae. This hypothesis implies, first, that the mean density of all clusters should be the same, and second, that they may be of any shape, including the spherical. He then tested his first conclusion on eight clusters reported by Hubble and Humason and on twenty-five studied by Shapley, and found a very satisfactory agreement with his theoretical expectation. The suggestion that the collapsing regions are the extragalactic nebulae led, in Lemaitre's hands, to a rather satisfactory answer to the question of the origin of the energy involved in the creation of stars within a nebula.

The purpose of this note is to point out that, so long as Lemaitre's con-



clusions as outlined in his papers have not been disproved, it cannot be seriously maintained that the existence of clusters of nebulae in any way contradicts the theory of the expanding universe, or the cosmological time scale associated with it.

<sup>1</sup> F. Zwicky, these PROCEEDINGS, 25, 604 (1939).

<sup>2</sup> G. Lemaitre, *Ibid.*, 20, 12 (1934).

Elaborations and further tests of these ideas were presented by Lemaitre in a paper read at the University of Notre Dame's symposium on cosmic physics, held in April, 1938. This paper has not, to the writer's knowledge, ever been published.

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## THE OPACITY OF EXTENDED STELLAR ATMOSPHERES

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1. In a recent paper<sup>1</sup> the expanding atmosphere of P Cygni was treated as a layer of gas which is transparent for radiation except within the absorption lines. The equivalent widths of the absorption lines furnished a measure of  $N_2H$ —the number of atoms per cm.<sup>2</sup> above the photosphere responsible for the lines. The intensities of the emission lines, on the other hand, furnished a measure of the quantity  $(1/W)N_eH$ , where  $W$  is the dilution factor and  $N_eH$  is the number of atoms per cm.<sup>2</sup> above the photosphere, in the upper level of each emission line. Adopting a relation of the Boltzmann type between  $N_2$  and  $N_e$ , we should, in principle, be able to derive  $W$ . The conclusion was that the emission lines are relatively too weak, and that, in consequence, the value of  $W$  was much larger than that derived from spectroscopic criteria, such as the relative intensities of lines arising from metastable and from normal levels.

2. The difficulty is removed if we abandon the assumption that the expanding shell in P Cygni is transparent for continuous radiation. There is, in fact, abundant evidence that the shell has a considerable optical thickness. If we compare P Cygni, 17 Leporis and HD 190073, we notice that these three stars show, with increasing clearness, the underlying spectrum of a stationary layer, which in HD 190073 and 17 Leporis gives rise to typical broad hydrogen absorption lines whose contours are produced by Stark effect. In 17 Leporis, moreover, we observe an undisplaced absorption line of Mg II 4481. There can be no doubt that the expanding shell of HD 190073 is almost perfectly transparent. That of 17 Leporis is already markedly affected by continuous absorption and emission in the shell. The broad hydrogen wings are too weak for class  $A_0$ , but the star cannot

be placed among the *B*'s, or among the later *A*'s, because we fail to observe undisplaced He lines and we also fail to observe strong undisplaced lines characteristic of the later subdivisions of class *A*. Nor can the underlying spectrum be classified as that of a normal giant, for in that case it would show a *strong* undisplaced line of Mg II 4481 and, in addition, strong undisplaced components of Fe II, Ti II, etc. Finally, in P Cygni there is no trace left of the undisplaced lines, or of the Stark wings of the hydrogen lines, and we must conclude that the optical thickness of the expanding shell is considerable.

A survey of the *Be* stars shows that, in general, when the outer shell gives rise to strong, narrow absorption lines of hydrogen, as in  $\phi$  Persei, the normal stellar lines of He and the broad wings of H are always very weak. This phenomenon is quite conspicuous, but its recognition has been delayed by the well-known correlation between the widths of the emission lines and the rotational broadening of the He lines.<sup>2</sup> The latter tends to make the He lines appear rather inconspicuous. It requires a comparison of the He lines in such *Be* stars as  $\phi$  Persei with normal stars having similar rotational velocities to ascertain the effect of the continuous absorption of the shell.

3. We may consider the atmosphere of a *Be* star to consist of: (1) a normal layer below a certain optical thickness,  $\tau_1 = \int_0^{\infty} \kappa \rho dz$ , where  $\kappa$  is the continuous absorption coefficient of the shell; and (2) of a tenuous shell above  $\tau_1$ . A stratified model of a stellar atmosphere has been treated by Eddington<sup>3</sup> and by Swings and Chandrasekhar.<sup>4</sup> Adopting the case of pure scattering ( $\epsilon = 0$ , in Eddington's notation) and setting

$$\begin{aligned} \lambda_{gr} &= \text{absorption coefficient of atoms within the line} \\ \kappa_{gr} &= \text{absorption coefficient in the continuous spectrum} \\ \eta &= \frac{\lambda}{\kappa} \end{aligned}$$

we find for the residual intensity within the line

$$H'/H = 1 - \frac{\sqrt{3}\eta}{2 \left\{ \left[ 1 + \eta + \frac{\sqrt{3}(1+\eta)}{2} \right] \sinh \sqrt{3}\tau_1 + \left[ \sqrt{1+\eta} + \frac{\sqrt{3}(1+\eta)}{2} \right] \cosh \sqrt{3}\tau_1 \right\}}.$$

Consider a point within the Stark-wing of a normal hydrogen line, as produced in the stationary layer below  $\tau_1$ . Let the intensity of this point in a star not surrounded by a shell be  $H'/H = 0.6$ . Then, for  $\tau > \tau_1$  we have  $\eta = 2$ . Above  $\tau_1$  we have, of course,  $\eta = 0$ . Numerical computations show that if  $\tau_1 = 0.1$ , then  $H'/H = 0.66$ . This is approximately correct for 17 Leporis. For P Cygni we must have  $\tau_1 \gtrsim 0.5$ .

These estimates rest solely upon the decrease of the intensities of absorp-

tion lines when seen through a shell. The question arises whether they are consistent with the known absorption coefficients and the known amounts of matter in the shells. Consider first the case of 17 Leporis. We have not yet been able to measure accurately the equivalent widths of the absorption lines. But since the hydrogen lines are similar to those of P Cygni, we may estimate  $N_2H = 10^{16}$ . In order to estimate the optical thickness, we must know the number of hydrogen atoms in the neutral state. Hence we apply the Boltzmann formula (and disregard possible departures from thermodynamic equilibrium):

$$\log \frac{N_2}{N} = -x_2 \frac{5040}{T} + \log \frac{g_2}{u}.$$

For 17 Leporis  $T = 10,000^\circ$ . This gives, approximately,

$$N = 10^{20}.$$

The continuous absorption coefficient per gram of neutral hydrogen,<sup>5</sup> for  $T = 10,000^\circ$ , and at  $\lambda 5000$  is

$$\kappa_{gr} = 20.$$

Since  $m_H = 1.7 \times 10^{-24} \text{ gr}$ , we find that the optical depth produced by  $10^2$  neutral hydrogen atoms at  $T = 10,000^\circ$  is

$$\tau_1 = 3 \times 10^{-3}.$$

We require  $\tau_1 = 10^{-1}$ . Hence, hydrogen is not sufficient to produce the required opacity, provided our estimate of  $N_2H$  is correct. The continuous absorption of the metals and of the free electrons may easily increase  $\tau_1$  to approximately  $10^{-2}$ . The remaining discrepancy—by a factor of about 10—is not sufficiently serious to cause concern, and there is at present no need to search for other sources of continuous absorption.

These considerations cannot be applied directly to P Cygni. Here the optical thickness of the shell is so great that our observational value of  $NH$  does not, even approximately, represent the number of atoms above  $\tau_1$ . Hence, we must proceed in a different manner. In the determination of  $N_2H$  we had assumed that the atmosphere is transparent and that re-emission can be neglected. Hence, we adjusted  $N_2H$  in such a manner that  $e^{-N_2H\lambda_{at}}$  correctly represented the observed equivalent width. A weak line was used. Hence

$$e^{-N_2H\lambda_{at}} \approx 1 - N_2H\lambda_{at}.$$

The stratified atmosphere must now be considered to contain absorbing atoms only above  $\tau_1$ . Therefore  $\eta = \eta_0$  when  $\tau < \tau_1$  and  $\eta = 0$  when  $\tau > \tau_1$ . The continuous absorption coefficient  $\kappa$  is constant for all values of  $\tau$ .

The corresponding solution of the problem of radiative transfer by Eddington<sup>3</sup> and by Swings and Chandrasekhar<sup>6</sup> gives<sup>7</sup>

$$H'/H = f(\eta, \tau_1) = 1 - \frac{\eta}{1 + \eta} \cdot \frac{\frac{q}{\sqrt{3}} \sinh q\tau_1 + \cosh q\tau_1 - 1}{\left(\frac{2}{q} + \frac{q}{\sqrt{3}}\right) \sinh q\tau_1 + \left(1 + \frac{2}{\sqrt{3}}\right) \cosh q\tau_1},$$

where  $q = \sqrt{3(1 + \eta)}$ .

Accordingly, we may write

$$NH\lambda_{at} = 1 - f(\eta, \tau_1).$$

But

$$\eta = \frac{\lambda_{gr}}{\kappa_{gr}} = \frac{\lambda_{at}\zeta}{\mu_{gr} m_H},$$

where  $\zeta$  is the ratio of the atoms in the lower level of the line to all atoms of the gas and  $\mu$  is the mean atomic weight of the unionized gaseous mixture.

We infer again from Unsöld's table<sup>5</sup> that for  $T = 25,000^\circ$  and  $\lambda = 5000$ , the continuous absorption coefficient per gram of neutral hydrogen is

$$\kappa_{gr} = 2 \times 10^6.$$

Let us consider a specific point on the contour of the absorption line produced by the shell, for which  $\eta = 1$ . Then

$$\lambda_{gr} = 2 \times 10^6.$$

If we are dealing with a shell consisting of pure hydrogen, then  $\zeta$  is given by the Boltzmann factor, provided  $\kappa_{gr}$  is expressed per gram of neutral hydrogen:

$$\zeta = 10^{-2}.$$

Accordingly,  $\lambda_{at} \approx 3 \times 10^{-17}$ . For the number of atoms we found<sup>8</sup>  $N_2H \approx 10^{15}$ . The problem is, therefore, to adjust  $\tau_1$  in such a manner that the following expression is fulfilled

$$3 \times 10^{-2} = 1 - f(\eta, \tau_1).$$

By means of trials we find, approximately,

$$\tau_1 = 0.05.$$

This is again too small, by a factor of 10. But we have allowed only for absorption in hydrogen. Other sources of continuous absorption will increase this value, and will probably be sufficient to remove the discrepancy.

The absorption lines of the shell are of the same order of strength as the normal absorption lines of a giant star of similar ionization. Hence, if the

absorbing properties of matter are the same in a normal giant reversing layer and in a shell, it is obvious that the optical depth  $\tau_1$  will be roughly 0.5—this being Unsöld's value<sup>9</sup> for the wings of normal absorption lines. Hence the discrepancies found for 17 Leporis and P Cygni would, if they were real, merely suggest greater values of  $\kappa$  than those which we have used.

4. The recognition of the existence of appreciable continuous absorption in the shells of P Cygni and 17 Leporis suggests an explanation of the relative weakness of the emission lines, mentioned in section 1. It is clear that the absorption lines will be favored if the emission lines come only from that part of the shell for which the optical thickness is less than that of the fictitious photosphere ( $\tau_1 < 1/3$ , according to Milne).

Another interesting consequence of our results is their bearing upon Kosirev's theory<sup>10</sup> of reddening in extended atmospheres. We know now that in P Cygni the opacity of the expanding shell is considerable. From the fact<sup>1</sup> that the dilution factor is of the order of 0.1, we conclude that the total thickness of the shell may be equal to the radius of the star. For such a layer it is no longer permissible to neglect the curvature—and the problem of radiative transfer must be solved along the lines suggested by Chandrasekhar<sup>11</sup> and by Kosirev.<sup>10</sup> The effect of reddening follows from this treatment.

It is probable that the same explanation holds in the case of  $\gamma$  Cassiopeiae. According to the observations of Greaves and Martin<sup>12</sup> the color temperature of this star dropped from 16,100°K. in 1926.78 to 9200°K. in 1937.72. This change was accompanied by the outburst of a shell which produced strong absorption lines,<sup>13</sup> and which must have had a considerable optical thickness.

The problem of the absorption lines is not seriously affected by the curvature of the shell. Chandrasekhar has derived a simple formula<sup>14</sup> for an infinite atmosphere. The case of a stratified curved atmosphere has not been investigated, but it is clear that the change will be negligible.

5. The question may arise whether the broad lines in 17 Leporis and  $\phi$  Persei really arise in the same star the outer shell of which produces the sharp lines. In the case of  $\phi$  Persei this question has been answered by an observation of Hynek<sup>15</sup> who has found from measurements of line displacements that the broad hydrogen wings and the sharp cores come from the same source. Another question is whether the outer shell envelops all parts of the photosphere, or whether we are dealing here with a phenomenon similar to that of the solar prominences. It is conceivable that the sharp lines are formed in a large mass of separate prominences, and that we are able to see the photosphere through the open spaces between the individual prominences. Observations of the sharp and very strong line Ca K in 17 Leporis show no appreciable intensity in the center. Hence the continuous spectrum of the normal photosphere is not superposed over the

violet-displaced line of the expanding shell. We infer that the shell is essentially unbroken, and that the broad hydrogen wings are seen through the shell and not between separate prominences.

<sup>1</sup> O. Struve and F. E. Roach, *Ap. Jour.*, **90**, 727 (1939).

<sup>2</sup> *Ap. Jour.*, **73**, 94 (1931).

<sup>3</sup> *M. N.*, **89**, 626 (1929); *The Internal Constitution of the Stars*, German Edition, pp. 424-425 (1928).

<sup>4</sup> *M. N.*, **97**, 33 (1936).

<sup>5</sup> A. Unsöld, *Die Physik der Sternatmosphären*, p. 121 (1938).

<sup>6</sup> *M. N.*, **97**, 32 (1936).

<sup>7</sup> The form of this equation is due to Mr. Ralph Williamson of the Yerkes Observatory.

<sup>8</sup> *Ap. Jour.*, **90**, 749 (1939).

<sup>9</sup> *Op. cit.*, p. 247.

<sup>10</sup> *M. N.*, **94**, 430 (1934).

<sup>11</sup> *Ibid.*, **94**, 444 (1934); *Russian Astr. Jour.*, **6** (6), 8 (1934).

<sup>12</sup> *Ibid.*, **98**, 434 (1938).

<sup>13</sup> Baldwin, *Ap. Jour.*, **89**, 256 (Plate 17) (1939).

<sup>14</sup> *M. N.*, **94**, 456 (1934).

<sup>15</sup> Unpublished.

## NORMAL ALGEBRAIC NUMBER FIELDS

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1. *Introduction.*—In this note we present a brief outline of investigations on the generalization of class field theory to arbitrary normal fields. A complete treatment of the results will be submitted for publication to the *Transactions of the American Mathematical Society*. In the sequel  $k$  denotes an algebraic number field of finite degree over the field of all rational numbers. Class field theory<sup>1</sup> deals with the description of abelian extensions over  $k$  in terms of classes of ideals in  $k$ . In other words, it is the analog of the theory of multiplicative functions on algebraic Riemann surfaces. It is possible to describe the class groups of the classical theory by means of the totality of all  $p$ -adic extensions  $k_p$  of  $k$ ;  $p$  denoting the prime divisors<sup>2</sup> of  $k$ .

2. *Ideal Algebras.*—Let  $H_p$  denote a class of normal simple algebras with index  $m_p$  over  $k_p$ .<sup>3</sup> A set  $\{H_p, \text{all } p\} = H$  shall be termed an *ideal algebra* if and only if  $m_p = 1$  for all but a finite number of prime divisors. If  $H^{(1)} = \{H_p^{(1)}\}$ ,  $H^{(2)} = \{H_p^{(2)}\}$ , then we define the product  $H^{(1)} \times H^{(2)}$  as  $\{H_p^{(1)} \times H_p^{(2)}\}$ . This definition implies that all ideal algebras form a group  $H$ . Now let  $K$  be a normal extension of degree  $n$  over  $k$  with



Galois group  $\Gamma = \{\sigma, \dots, \tau\}$ . Let  $n_p = [K_P : k_p]$ , where  $P$  is a typical prime divisor of  $p$  in  $k$ . We say that  $K$  splits an ideal algebra  $H$  if  $n_p \equiv 0 \pmod{m_p}$  for all prime divisors  $p$ . Thus  $H$  contains as a subgroup the class group of all normal simple algebras  $S/k$  which are split by  $K$ .

In order to describe our generalization of class field theory we term "algebras"  $H$  or  $S$  relatively prime to a divisor  $M = \prod P^a$  (in  $K$ ) if no  $P \subset k$  is a ramification divisor<sup>4</sup> of  $H$  or  $S$ , respectively. The group of all ideal algebras that are relatively prime to  $M$  can be called  $H_M$ . It contains the group  $S_M$ . Our investigations deal with the evaluation of the index

$$[H_M : S_M], \quad M = \prod_P P^{a(P)}$$

for arbitrary normal fields  $K/k$  and properly selected divisors  $M$  depending on the structure of  $K$  relative to  $k$ . If the field  $K$  is specialized to a cyclic field  $Z$  then the general index reduces to the index of the class group associated to  $Z$  in  $k$  by the class field theory.

3. *Theory of Primary Factor Sets.*—In order to actually evaluate the index  $[H_M : S_M]$  we use a device first developed by Artin in some unpublished work.<sup>5</sup> Let  $\Gamma_I = \{\sum c_\sigma \sigma\}$  be the group ring of  $\Gamma$  with coefficients  $c_\sigma$  in the ring  $I$  of all integers. Suppose that  $P$  is an arbitrary prime divisor of  $K$ ,  $\Delta_P$  the associated decomposition group. The conjugates of  $P$  are obtained by applying to  $P$  substitutions  $\lambda \in \Gamma$  which are representatives of  $\Gamma$  modulo  $\Delta_P$ . We then consider the infinite cyclic group  $\{P\}$  generated by  $P$  under the operators of  $\Gamma_I$ . A  $p$ -primary factor set  $F\{P\}$  is a set of  $n^2$  ideals  $P^{a_{\sigma,\tau}}$  in  $\{P\}$  with exponents  $a_{\sigma,\tau}$  in  $\Gamma$  which satisfy the  $n^2$  conditions

$$a_{\sigma,\tau} + a_{\sigma\tau,\rho} = \sigma a_{\tau,\rho} + a_{\sigma,\tau\rho}.$$

These associativity relations imply that, for suitable elements  $b_\sigma$ ,

$$na_{\sigma,\tau} = b_\sigma + \sigma b_\tau - b_{\sigma\tau}, \text{ i.e.,}$$

$$(P^{a_{\sigma,\tau}})^n = P^{b_\sigma} P^{\sigma b_\tau} P^{-b_{\sigma\tau}},$$

a factor set of transformation quantities.<sup>6</sup> All factor sets of transformation quantities form a subgroup  $T\{P\}$  of  $F\{P\}$ . The theory of group characters yields that the index of this subgroup is

$$[F\{P\} : T\{P\}] = [\Delta_P : \Delta_P'],$$

where  $\Delta_P'$  denotes the commutator group of  $\Delta_P$ . Thus, if  $p = P \subset k$  is unramified in  $K$  we find

$$[\Delta_P : \Delta_P'] = f(p);$$

$f(p)$  denoting the relative residue class degree of a typical  $P$  dividing  $p$ . By the fundamental theorem of ideal theory we can collect factor sets  $F\{P\}$  to factor sets  $F\mathfrak{A}$  of arbitrary ideals  $\mathfrak{A}$  in  $K$ .

A factor set  $A_{\sigma, \tau}$  of numbers from  $K$  always determines a crossed product  $(K/k, \Gamma, A_{\sigma, \tau})$  which is a normal simple algebra over  $k$ . In terms of these algebras we next define certain factor sets  $(B_{\sigma, \tau})$  of principal ideals which correspond to the principal ideals generated by norm residues in the cyclic theory. The  $B_{\sigma, \tau}$  are all those factor sets defined by the restriction that each  $B_{\sigma, \tau}$  is prime to the divisor  $M$ , and the restriction on the corresponding crossed product that<sup>7</sup>

$$(K/k, \Gamma, B_{\sigma, \tau})_p \sim k_p, \text{ for all } p \text{ ramified in } K/k.$$

Let  $F_M(A_M)$  denote the group of all factor sets  $(B_{\sigma, \tau})$ . Using the theory of rational algebras and the existence theorem for prime divisors with given Frobenius symbol we arrive at<sup>8</sup>

$$\begin{aligned} [H_M : S_M] &= [F\mathfrak{A}_M : T\mathfrak{A}_M \cdot F_M(A_M)] \\ &= \text{l. c. m. of all orders of elements } \sigma \in \Gamma. \end{aligned}$$

The definition of the  $B_{\sigma, \tau}$  also determines the divisor  $M$  for which we must consider  $H_M, S_M$ . We observe that these results yield ordinary class field theory only for cyclic extensions. Namely, if  $\Gamma$  is the fours group then  $[H_M : S_M] = 2$ , whereas the inversion theorem of class field theory yields the value 4 for the index of the class group.

4. *Determination of  $[F\mathfrak{A}_M : T\mathfrak{A}_M \cdot F_M(A_M)]$  by Means of Index Reduction.*—Another way of evaluating the index in question is to generalize the proof of the classical inversion Theorem.<sup>9</sup> Lack of space does not permit us to sketch the important steps of our reduction. At various stages of the process we have to consider arbitrary normal extensions  $K_P/k_p$ . Let  $E$  denote the group of all units in  $K_P$  which are congruent to 1 modulo  $P$ . If  $\zeta$  and  $\eta$  range over the decomposition group  $\Delta_P$ ,  $E_{\zeta, \eta}$  will denote a corresponding factor set of units from the group  $E$ , while  $F_\Delta E$  denotes the group of all such factor sets. We define the local conductor  $C(P)$  of  $K_P/k_p$  as the least power of  $P$  such that

$$E_{\zeta, \eta} \equiv 1 \pmod{C(P)}$$

implies

$$(K_P/k_p, \Delta_P, E_{\zeta, \eta}) \sim k_p.$$

It is readily seen that  $C(P)$  is the unit divisor if  $P/p$  is not ramified. If  $P/p$  has no higher ramifications, then  $K_P$  is cyclic of degree prime to  $P$  over its unramified subfield and  $C(P) = p$ . The divisor  $\Pi_P C(P)$  can be chosen as the  $M$  of the preceding discussion. Thus,  $M$  can be selected to be an invariant divisor of  $K/k$ . The intersection  $R(M)$  of all groups  $E$  defined above for all  $P$  yields the generalization of the ray used in class field theory. Local class field theory yields

$$[FE:TE] = e(p),$$

where  $e(p)$  is the ramification degree of  $p$  in a typical  $K_p$ .

Moreover, we require information on factor sets  $U_{s,r}$  of units  $U$  in  $K$ . Applying the methods used in section 3 we find

$$[FU^* : TU^*] = [\Gamma : \Gamma']^r 2^\rho$$

for the Herbrand subgroup  $U^*$  of  $U$ .<sup>10</sup> Here  $\Gamma'$  denotes the commutator group of  $\Gamma$ ,  $r$ , the number of basal units  $u = U \cap k$  which lie in the ground field  $k$ ,  $\rho$  the number of real (infinite) prime divisors of  $k$  that are ramified in  $K$ . In order to evaluate  $[FU : TU]$  we make repeated use of Herbrand's Lemma and find that the essential index of this reduction is

$$[FU : FU^*] = [U : U^*]^{s-1} [U \cap k : U^* \cap k] \cdot L$$

where  $L$  denotes a rather complicated factor of reduction. Examples show that  $L$  can be different from unity, although in the cyclic case the analogous index can be proved to be one, by the Herbrand Lemma on groups. Combining the various results we ultimately find

$$[F\mathfrak{U}_M : T\mathfrak{U}_M \cdot F_M(A_M)] = [FA_M : F(A_M)] [\Gamma : \Gamma'] h_K^{1-n} h_k^{-1} L^{-1},$$

where  $L$  and  $\Gamma'$  are defined as above, while

$$h_K = [\mathfrak{U} : (A)], \quad h_k = [\mathfrak{U} \cap k : (A \cap k)]$$

are the respective class numbers of  $K$  and  $k$ . (Here  $A$  denotes the group of all non-zero numbers of  $k$ ,  $(A)$ , the group of all principal ideals of  $K$ .)

5. *Generalized Artin Symbol and Norm Residue Symbol.*—Finally, by means of our results, we can define a generalization of the classical symbols of class field theory. They yield formal implications of our general theory, which is based on the notions of ideal algebras and classes of algebras. These symbols can be used to describe the law of decomposition of unramified prime divisors.<sup>11</sup>

<sup>10</sup> C. Chevalley, "Généralisation de la théorie du corps de classes pour les extensions infinies," *Jour. Math. pures et appliquées*, ser. 9, 15, 359–371 (1936); A. Weil, "Remarques sur les résultats récents de C. Chevalley," *C. R.*, Paris, 203, 1208 (1936); A. Weil, "Zur algebraische Theorie der algebraischen Funktionen," *Jour. reine ang. Math.*, 179, 129–133 (1938).

<sup>11</sup> As usual, a prime divisor of  $k$  is simply a valuation of  $k$ . These valuations are of two types: "finite" and "infinite." Each prime ideal  $\mathfrak{p}$  gives a finite prime divisor, for which the valuation function is  $V_{\mathfrak{p}}(\alpha)$ , the exact exponent to which  $\alpha$  is divisible by  $\mathfrak{p}$ . The infinite prime divisors correspond to archimedean valuations of  $k$  given by the various subfields of the complex field conjugate to  $k$ .

<sup>12</sup> For general information, see: A. A. Albert, "Structure of Algebras," *Am. Math. Soc. Col. Pub.*, 24 (1939).

<sup>13</sup>  $\mathfrak{p}$  is a ramification divisor of  $H$  if  $m_{\mathfrak{p}} \neq 1$ , and conversely.

<sup>14</sup> M. Hall, "Group Rings and Extensions I," *Ann. Math.*, 39, 220–234 (1938); T.

Nakayama, "Ueber die Beziehungen der Faktorensystemen und der Normenklassengruppe eines galoisschen Erweiterungskörpers," *Math. Ann.*, **112**, 85-91 (1935).

<sup>6</sup> If  $R$  is any multiplicative abelian group with the operators of  $\Gamma$ , then  $FR$  will denote the group of all factor sets  $R_{\sigma,\tau}$  with components  $R_{\sigma,\tau}$  in  $R$  and subscripts  $\sigma, \tau$  ranging over  $\Gamma$ . In like manner  $TR$  designates the group of all transformation sets  $R_{\sigma}R_{\tau}^{\sigma}/R_{\sigma,\tau}$  formed from vectors  $R_{\sigma}$  over the group  $R$ .

<sup>7</sup> E. Noether, "Der Hauptgeschlechtssatz für relativgaloissche Zahlkörper," *Math. Ann.*, **108**, 411-419 (1932).

<sup>8</sup> Here, as previously, a subscript " $M$ " signifies that one is to consider only those elements prime to  $M$  in the group concerned.

<sup>9</sup> C. Chevalley, "Sur la theorie du corps de classes dans les corps finis et les corps locaux," *Jour. Fac. Sci., Univ. Tokyo*, **2**, 365-476 (1933); H. Hasse, Mimeographed lecture notes on Klassenkörpertheorie, Marburg, 1933.

<sup>10</sup> J. Herbrand, "Nouvelle démonstration et généralisation d'un théorème de Minkowski," *C. R., Paris*, **191**, 1282 (1930).

<sup>11</sup> R. Brauer, H. Hasse and E. Noether, "Beweis eines Hauptsatzes in der Theorie der Algebren," *Jour. reine angewandte Math.*, **167**, 399-404 (1932).

## ON AN ERGODIC ANALYSIS OF THE REMAINDER TERM OF MEAN MOTIONS

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Let  $a_1, \dots, a_n$  be positive constants for which  $b_1 + \dots + b_k \neq b_{k+1} + \dots + b_n$ , ( $k = 1, 2, \dots, n-1$ ) if  $(b_1, \dots, b_n)$  is any permutation of  $(a_1, \dots, a_n)$ . Denote by  $z(t)$  the trigonometric sum

$$z(t) = \sum_{j=1}^n a_j e(\lambda_j t + \phi_j^0), \quad (e(u) = \exp 2\pi i u),$$

and suppose that the frequencies  $\lambda_1, \dots, \lambda_n$  are fixed and linearly independent, while the initial phases  $\phi_1^0, \dots, \phi_n^0$  are arbitrary on the  $n$ -dimensional torus  $T: 0 \leq \phi_j^0 < 1$ . One can exclude from  $T$  a  $(n-1)$ -dimensional  $\phi^0$ -hypersurface in such a way that for the remaining points of  $T$  the function  $z(t)$  does not vanish for  $-\infty < t < +\infty$ ; so that the pair of conditions

$$e(\psi(t)) = z(t)/|z(t)|; \quad 0 \leq \psi(0) < 1,$$

determines a unique regular function  $\psi$  of  $t$  along the  $t$ -axis. By an application of Birkhoff's ergodic theorem,<sup>1</sup> it was recently shown<sup>2</sup> that  $\psi(t)$  has, for almost all choices of the initial phases on  $T$ , a mean motion

$$\mu = \lim_{t \rightarrow \infty} \psi(t)/t, \text{ i.e., } \mu = M\{\psi'(t)\}, \text{ where } M\{g(t)\} = \lim_{v \rightarrow \infty} v^{-1} \int_0^v g(t) dt$$

(and  $\psi'(t) = d\psi(t)/dt$ ); so that the arcus  $\psi(t)$  may be analyzed into a secular term  $\mu \cdot t$  and a remainder term  $\omega(t) = \psi(t) - \mu \cdot t$  of lower order.

In view of the applications, one would expect that the remainder term  $\omega(t)$  admits of an anharmonic analysis, as tacitly assumed by the astronomers. However, the existence of such an analysis never was mathematically established (except in Lagrange's case  $a_1 > a_2 + a_3 + \dots + a_n$ , in which case  $\omega(t)$  turned out to be uniformly almost periodic<sup>3</sup>). The object of this note is to fill in this gap to some extent, by proving for  $\lambda \neq 0$  the existence of the Fourier averages  $F(\lambda) = M\{e(-\lambda t)\omega(t)\}$ ,  $-\infty < \lambda < +\infty$ , of  $\omega(t)$ , if, as before, a set of zero measure on  $T$  is excluded. The proof consists of a refinement of the considerations applied loc. cit.<sup>2</sup> In fact, Birkhoff's ergodic theorem will be applied, not once but infinitely often. What is actually proved in this manner is the almost periodicity ( $B$ ) of  $\omega'(t)$ . The existence of  $F(\lambda)$  then follows from the existence of  $G(\lambda) = M\{e(-\lambda t)\omega'(t)\}$  and from the relation

$$2\pi i F(\lambda) = G(\lambda)/\lambda, \quad (-\infty < \lambda < +\infty, \lambda \neq 0; G(0) = 0),$$

which may be established by using the existence of  $\mu$ .

It will also follow that  $G(\lambda)$  is, for fixed  $\lambda$ , homogeneous and linear in the given frequency  $\lambda_1, \dots, \lambda_n$  of  $z(t)$ , and that  $G(\lambda) = 0$  unless  $\lambda$  is of the form  $m_1\lambda_1 + \dots + m_n\lambda_n$ , where  $m_j = 0, \pm 1, \pm 2, \dots$  for  $j = 1, \dots, n$ . Hence, it is seen from the preceding connection between  $G$  and  $F$ , that the Fourier averages of  $\omega(t)$  involve the small divisors of classical celestial mechanics,<sup>4</sup> or, equivalently, the Diophantine problematics of irrational rotation numbers in the general stability problem of Birkhoff.<sup>5</sup> Thus, the Fourier analysis of the remainder term  $\omega(t)$  of  $\psi(t) = \mu \cdot t + \omega(t)$  automatically leads, at least formally, to the central unsolved problem of general dynamics. This might be connected with the circumstance that, although the derivative  $\omega'(t) = \psi'(t) - \mu$  turns out to be almost periodic ( $B$ ), the almost periodicity ( $B$ ) of  $\omega(t)$  itself will remain undecided.

First, if  $T$  denotes the  $n$ -dimensional torus  $0 \leq \phi_j < 1$ , ( $j = 1, \dots, n$ ), and if the amplitudes  $a_j$  and the frequencies  $\lambda_j$  of  $z(t)$  are fixed, then, on placing

$$\chi(\phi_1, \dots, \phi_n) = \text{real part of } \sum_{j=1}^n \lambda_j a_j e(\phi_j) / \sum_{j=1}^n a_j e(\phi_j),$$

one obtains a function  $\chi$  of the position  $(\phi_1, \dots, \phi_n)$  on  $T$ . It was proved loc. cit.<sup>2</sup> that  $\chi$  is integrable ( $L$ ) over  $T$ . On expressing  $\exp i\alpha$  in terms of  $\cos \alpha$  and  $\sin \alpha$ , one readily sees that  $\chi(-\phi_1, \dots, -\phi_n) = \chi(\phi_1, \dots, \phi_n)$ . Hence, the Fourier series ( $L$ ) of  $\chi$  on  $T$  is of the form

$$\chi(\phi_1, \dots, \phi_n) \sim \sum A(m_1, \dots, m_n) \cos 2\pi(m_1\phi_1 + \dots + m_n\phi_n),$$



where the  $n$  summation indices  $m_1, \dots, m_n$  run from  $-\infty$  to  $+\infty$ . (Since  $\chi$  is homogeneous and linear in  $(\lambda_1, \dots, \lambda_n)$ , the same holds for each of the Fourier constants  $A$ .) Let  $k$  denote the collection  $(k_1, \dots, k_n)$  of  $n$  positive integers  $k_j$ , and  $\sigma_k = \sigma_k(\phi_1, \dots, \phi_n)$  the  $n$ -fold trigonometric polynomial which is the  $k$ th partial sum of the Fourier series of  $\chi$ . Then, since  $\chi$  is integrable ( $L$ ), one has

$$\int_T |\chi(\phi_1, \dots, \phi_n) - \sigma_k(\phi_1, \dots, \phi_n)| dT \rightarrow 0 \text{ as } k \rightarrow \infty,$$

if  $k \rightarrow \infty$  means that  $k_j \rightarrow \infty$ ;  $j = 1, \dots, n$ .

Consider the cyclic transformation group

$$\phi_1 = \lambda_1 t + \phi_1^0, \dots, \phi_n = \lambda_n t + \phi_n^0; \quad -\infty < t < +\infty.$$

This group of transformations of  $T$  into itself is metrically transitive in view of the linear independence of  $\lambda_1, \dots, \lambda_n$  (Kronecker-Weyl). Furthermore, the function  $|\chi - \sigma_k|$  on  $T$  is integrable ( $L$ ) for every fixed  $k$ . But  $\sigma_k(\phi_1, \dots, \phi_n)$  becomes, in virtue of the cyclic group, an almost periodic polynomial  $s_k(t) = \sigma_k(\lambda_1 t + \phi_1^0, \dots, \lambda_n t + \phi_n^0)$ , while the definition of the function  $\chi$  on  $T$  implies that  $\chi(\lambda_1 t + \phi_1^0, \dots, \lambda_n t + \phi_n^0)$  is identical with the derivative  $\psi'(t)$  of the angular function  $\psi(t)$ , defined in the introduction. It follows therefore from the ergodic theorem of Birkhoff that, if  $k$  is arbitrarily fixed and if the initial phase  $(\phi_1^0, \dots, \phi_n^0)$  on  $T$  is chosen arbitrarily but not on a certain zero set (which, if it exists at all, might depend on  $k$ ), then the time average  $M\{|\psi'(t) - s_k(t)|\}$  exists and is identical with the space average of  $|\chi - \sigma_k|$  over  $T$ . But, as was pointed out before, this space average tends to zero as  $k \rightarrow \infty$ ; so that, since for each of the integral indices  $k = (k_1, \dots, k_n)$  only a zero set of initial phases is excluded, the relation

$$M\{|\psi'(t) - s_k(t)|\} \rightarrow 0, \quad k \rightarrow \infty,$$

holds whenever the initial phase  $(\phi_1^0, \dots, \phi_n^0)$  is not in a zero set of  $T$ .

Since  $s_k(t)$  is an almost periodic polynomial, it follows that  $\psi'(t)$  is almost periodic ( $B$ ) for almost all choices of the initial phase. Furthermore, it is clear from the definition of  $s_k(t)$  that the anharmonic Fourier analysis ( $B$ ) of  $\psi'(t)$  may be obtained by writing  $\lambda_1 t + \phi_1^0, \dots, \lambda_n t + \phi_n^0$  for  $\phi_1, \dots, \phi_n$  in the Fourier series ( $L$ ) of the periodic function  $\chi(\phi_1, \dots, \phi_n)$ .

Finally, the passage from  $\omega'(t) = \psi'(t) - \mu$  to  $\omega(t) = \psi(t) - \mu t$ , as described in the introduction, requires merely a cautious application of partial integrations on the average.

It was suspected loc. cit.<sup>2</sup> (p. 263) that, as far as the existence of the constant  $\mu$  is concerned, the exceptional zero set of the ergodic theorem is vacuous, and Weyl<sup>6</sup> has succeeded in proving, among other things, that such is actually the case. However, it remains undecided whether or not



the same is true in case of the finer problem of the present note. On the other hand, there is no difficulty at all in transferring the above considerations to the case where the frequency  $\lambda_1, \dots, \lambda_n$  of  $z(t)$  are not linearly independent.<sup>7</sup>

<sup>1</sup> G. D. Birkhoff, *Proc. Nat. Acad. Sci.*, 17, 656–660 (1931).

<sup>2</sup> P. Hartman, E. R. van Kampen, and A. Wintner, *Amer. Jour. Math.*, 59, 261–269 (1937).

<sup>3</sup> A. Wintner, *Rend. R. Accad. Naz. Lincei*, (6) 11, 464–467 (1930).

<sup>4</sup> Cf. A. Wintner, *Math. Zeit.*, 31, 434–440 (1930) and *Amer. Jour. Math.*, 59, 801 (1937).

<sup>5</sup> Cf. G. D. Birkhoff, *Ann. Inst. Poincaré*, 2, 369–386 (1932).

<sup>6</sup> H. Weyl, *Amer. Jour. Math.*, 60, 889–896 (1938).

<sup>7</sup> Cf. loc. cit., reference 2, 263; H. Weyl, *Ibid.*, 61, 143–148 (1939)

## GROUPS WHICH CONTAIN LESS THAN FOURTEEN PROPER SUBGROUPS

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The earliest fundamental theorem relating to the development of the groups of finite order was announced in an incomplete form by J. L. Lagrange (1736–1813) and asserts that the order of a group is divisible by the order of each of its subgroups. The work of J. L. Lagrange related directly only to the subgroups of the symmetric group but the method of the proof in this special case is easily extended to the general case and hence the general theorem has sometimes been called Lagrange's theorem relating to group theory. It may be noted that this was just about one hundred years earlier than the announcement of Sylow's theorem in 1872, which incorrectly is said to be the first to be proved among a set of closely related theorems on the structure of finite groups, in the *Encyclopaedia Britannica*, volume 10, page 914 (1938). The present article is one of a series of recent contributions along primitive lines on the subject of finite groups.

The groups which contain less than twelve proper subgroups were determined in these PROCEEDINGS, 25, 540–543 (1939). If an abelian group contains exactly twelve proper subgroups its order cannot be divisible by as many as three distinct prime numbers since such a group is the direct product of its Sylow subgroups. If its order is divisible by two distinct prime numbers it is the cyclic group of order  $p_1 p_2^6$ ,  $p_1$  and  $p_2$  being distinct prime numbers. If it is a non-cyclic prime power group one of its invariants is a prime number  $p$ , and therefore the number of its proper subgroups

is  $(k-1)(p+1)$ ,  $p^k$  being the order of the group. Hence there is one and only one such group of each of the following orders: 32, 81, 125, 121. These correspond to the following pairs of values of  $p$  and  $k$ , respectively: 2, 5; 3, 4; 5, 3; 11, 2. When the group is cyclic its order is  $p^{13}$ . Hence *there are exactly six abelian groups which separately involve twelve proper subgroups*. Two of these are cyclic and consist of infinite systems of groups.

If  $G$  is a non-abelian prime power group which contains exactly twelve proper subgroups its order cannot be 16 and if its order is 32 it is the group of this order which contains exactly two non-invariant subgroups. Hence there is one and only one non-abelian group which contains exactly twelve proper subgroups and has an order which is of the form  $2^m$ . If a non-abelian group whose order is of the form  $3^m$  contains exactly twelve proper subgroups it contains an invariant subgroup of order 27 which involves operators of order 9. This subgroup involves eight proper subgroups. Hence it must contain operators of order 27 and be the group of order 81 which involves three and only three non-invariant subgroups. From similar considerations it results that if a non-abelian group whose order is of the form  $5^m$  contains exactly twelve proper subgroups it is the group of order 125 which contains exactly five non-invariant subgroups of order 5. Since a non-abelian group whose order is a prime power of a larger prime number than 5 cannot contain exactly twelve proper subgroups it has been proved that *there are exactly three non-abelian prime power groups which separately contain exactly twelve proper subgroups*. The orders of these three groups are 32, 81 and 125, respectively, and they are all conformal with abelian groups.

When the order of a non-abelian group  $G$  which contains exactly twelve proper subgroups is divisible by two distinct prime numbers the larger of these prime numbers is less than 13, for  $G$  is not the direct product of its Sylow subgroups. If the larger of these prime numbers is 11 then  $G$  contains only one subgroup of order 11 and its order is either 22 or 55, being either the dihedral group of order 22 or the semi-metacyclic group of order 55. If the larger of the two prime factors of the order of  $G$  is 7 then  $G$  contains only one subgroup of order 7 and it is formed by establishing a 7, 4 isomorphism between the dihedral group of order 14 and the cyclic group of order 8 or by establishing a 7, 9 isomorphism between the semi-metacyclic group of order 21 and the cyclic group of order 27. If the larger of the two prime factors of the order of  $G$  is 5 then  $G$  contains an invariant subgroup of order 5 and is formed by a 5, 8 isomorphism between the dihedral group of order 10 and the cyclic group of order 16, or it is the metacyclic group of order 20.

If an abelian group contains exactly thirteen proper subgroups its order is divisible by at most two distinct prime numbers and the cyclic group of order  $p_1^2 p_2^4$ ,  $p_1$  and  $p_2$  being distinct prime numbers, is the only group whose

order is divisible by two distinct prime numbers which has exactly thirteen proper subgroups. The direct product of the four group and the cyclic group of order  $p^2$ ,  $p$  being an arbitrary odd prime number, is the only non-cyclic abelian group which contains exactly thirteen proper subgroups and has an order which is divisible by two distinct prime numbers. If a prime power abelian group contains exactly thirteen proper subgroups it is the group of order 16 and of type 2, 2 if it has two invariants and the cyclic group of order  $p^{14}$  if it has only one invariant. Hence there are four and only four abelian groups which separately involve thirteen and only thirteen proper subgroups. Three of these are infinite systems of groups while one is an individual group.

There are two non-abelian groups of order 16 which separately contain exactly thirteen proper subgroups. One of these is obtained by extending the cyclic group of order 8 by operators which transform each of its operators into the third power and the other is obtained by extending the abelian group of type 2, 1 by an operator of order 4 which transforms each of its operators into its inverse but has a different square than its operators of order 4. If a non-abelian group of order 32 would contain exactly thirteen proper subgroups it could not involve a cyclic group of order 16 and therefore each of its subgroups of order 16 would contain at least nine proper subgroups. Hence there are two and only two non-abelian groups which separately contain thirteen proper subgroups and have an order which is a power of 2. It is easy to see that a non-abelian group whose order is a power of an odd prime number cannot contain exactly thirteen proper subgroups and hence there are two and only two prime power non-abelian groups which separately involve exactly thirteen proper subgroups.

If the order of a non-abelian group which contains exactly thirteen proper subgroups is divisible by just two distinct prime numbers these prime numbers are 2 and 3, respectively. There are four Sylow subgroups whose order is a power of 3 in such a group, because if  $G$  contained only one such Sylow subgroup this subgroup could not be cyclic and of order as large as 9 because the dihedral group of order 18 contains fourteen proper subgroups. It could not be of order 3 since the corresponding quotient group would have for its order a power of 2 and could not be cyclic because  $G$  contains an odd number of proper subgroups. It could not be non-cyclic because there would be a subgroup of index 2 under  $G$  which would be the direct product of the group of order 3 and an abelian group whose order is a power of 2. As this is impossible  $G$  contains four subgroups whose order is a power of 3 and transforms them according to the tetrahedral group.

If these four subgroups are of order 9 they are cyclic and  $G$  is formed by a 4, 3 isomorphism between the tetrahedral group and the cyclic group of order 9. If the given four Sylow subgroups are of order 3 they generate the non-twelve group of order 24 which is known to contain thirteen proper

subgroups. The order of  $G$  cannot be divisible by as many as three distinct prime numbers since  $G$  could not be the direct product of two groups and a non-abelian group cannot contain exactly five proper subgroups. A simple group of composite order could not contain exactly thirteen proper subgroups since its order could not be divisible by more than two distinct prime numbers for there would be more than one Sylow subgroup of each order. The smallest possible order of  $G$  would be the product of powers of 2, 3, 5 and hence there would be at least  $3 + 4 + 6$  Sylow subgroups, but there would also be subgroups which would not be Sylow subgroups since if there were only 3 subgroups whose orders would be powers of 2 they would be transformed according to a group of degree 3 and hence  $G$  could not be simple. If the order of  $G$  would be divisible by as many as three distinct prime numbers the largest of these numbers would be at least equal to 5. This is impossible, and hence it results that *there are exactly eight different groups which separately contain thirteen proper subgroups*. The four non-abelian groups which have this property consist of two groups of order 16, one of each of the orders 24 and 36.

## ON THE DECOMPOSITION OF TRANSITIVE PERMUTATION GROUPS GENERATED BY THE SYMMETRIC GROUP

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1. *Introduction.*—The double cosets  $H_\alpha S H_\alpha$  into which the symmetric group  $G$  of degree  $n$  is decomposed with respect to a subgroup  $H_\alpha$  are associated in an interesting way with the irreducible representations  $\{\gamma\}$  which occur as components in the reduction of the permutation group  $G_H(\alpha)$ . The subgroup  $H = H_\alpha$  is taken to be the direct product of symmetric groups of orders  $\alpha_1, \alpha_2, \dots, \alpha_n$ ; and  $G_H(\alpha)$  is the transitive permutation group on the cosets  $H_\alpha S_i$  which orders to an element  $R$  of  $G$  the permutation  $H_\alpha S_i \rightarrow H_\alpha S_i R = H_\alpha S_j$ . If we restrict  $R$  to the elements of  $H_\alpha$ , the cosets which are then permuted among themselves form the aggregates  $H_\alpha S_i H_\alpha$  called double cosets. Now if  $G_H(\alpha)$  is written as a group of permutation matrices and completely reduced, and if  $\mu_\gamma^\alpha$  denotes the multiplicity of the irreducible representation  $\{\gamma\}$  in this reduction, then we have

$$G_H(\alpha) = \sum_{\gamma} \mu_\gamma^\alpha \cdot \{\gamma\}. \quad (1)$$

The number of Hermitian invariants of this group is equal on the one hand to the number of double cosets  $H_\alpha S H_\alpha$  and on the other hand to the sum

of the squares of the multiplicities  $\mu_\gamma^\alpha$  of the irreducible components  $\{\gamma\}$ , so we have

$$\sum_{\gamma} (\mu_\gamma^\alpha)^2 = \text{number of double cosets } H_\alpha S H_\alpha. \quad (2)$$

We propose to prove the following theorems.

**THEOREM I.**—The double cosets  $H_\alpha S H_\alpha$  of the symmetric group  $G$  (with respect to a subgroup  $H_\alpha$  which is the direct product of symmetric groups) can be displayed in a series of squares, one for each irreducible component  $\{\gamma\}$  of  $G_H(\alpha)$ , so that each square shall have its  $(\mu_\gamma^\alpha)^2$  double cosets so arranged that inverse double cosets are symmetrically placed with respect to the diagonal, and self-inverse double cosets occupy the diagonal positions.

**THEOREM II.**—The number of self-inverse double cosets  $H_\alpha S H_\alpha$  is equal to the number of irreducible components of  $G_H(\alpha)$ , each counted according to its multiplicity.

2. *Partitions and Tableaux.*—Each partition  $[\alpha]$  of an integer  $n$  into a sum of positive integers,

$$\alpha_1 + \alpha_2 + \alpha_3 + \dots + \alpha_r = n, \alpha_1 \geq \alpha_2 \geq \alpha_3 \geq \dots \geq \alpha_r > 0, \quad (3)$$

may be associated with an  $[\alpha]$ -tableau having  $\alpha_i$  dots (or symbols, or digits) in the  $i$ th row,  $i = 1, 2, \dots, r$ . Frobenius and others have associated such tableaux with sets of conjugate elements and with irreducible representations of the symmetric group  $G$  of degree  $n$ . We may further associate this tableau both with the subgroup  $H_\alpha$  of  $G$  which permutes symmetrically among themselves the symbols in each of the rows of the tableau, and also with the transitive permutation group  $G_H(\alpha)$  which gives a reducible linear representation of the symmetric group.

Following Littlewood, Richardson<sup>1</sup> and others, G. deB. Robinson<sup>2</sup> has used these tableaux in a study of the decomposition of the direct product of irreducible representations of the symmetric group. His representation of the elements of the symmetric group in a series of squares corresponding to the irreducible representations suggested the theorems of this paper about the double cosets  $H_\alpha S H_\alpha$ . For when the subgroup  $H_\alpha$  is the identity subgroup, the double cosets become the elements themselves, and his diagram becomes a special case of ours.

3. *Standard Tableaux and Double Cosets.*—We shall use the expression “normal  $[\alpha]$ -sequence” to denote a sequence of  $\alpha_1$  1’s,  $\alpha_2$  2’s,  $\alpha_3$  3’s,  $\dots$ ,  $\alpha_r$   $r$ ’s, in that order, and the expression “normal  $[\alpha]$ -tableau” to denote the same sequence arranged in  $r$  rows to form an  $[\alpha]$ -tableau. The substitution  $x_i \rightarrow x_j$  operating on a sequence or tableau is to be thought of as carrying the symbol in the  $i$ th position into the  $j$ th position. The subgroup  $H_\alpha$  of  $G$  obviously leaves a normal  $[\alpha]$ -sequence (or tableau) invariant, and each



element of a coset  $H_\alpha S$  transforms it into the same deranged  $[\alpha]$ -sequence (or tableau). The substitutions from the double coset  $H_\alpha S H_\alpha$  shuffle among themselves the digits or symbols of a given row of the deranged tableau corresponding to  $H_\alpha S$ , but they all leave the same number  $c_j^i(S)$  of digits  $j$  in the  $i$ th row. A particular substitution of  $H_\alpha S H_\alpha$  can be found which will arrange the digits in non-increasing order within each row, and we shall suppose that  $S$  itself is so chosen. The resulting tableau will be called a standard  $[\alpha]$ -tableau, and the corresponding sequence obtained by writing the successive rows of this tableau in a single line, separated by commas, will be called a standard  $[\alpha]$ -sequence. Both will be denoted by  $[\alpha]^S$ . There is thus a one-to-one correspondence between standard  $[\alpha]$ -sequences  $[\alpha]^S$  and double cosets  $H_\alpha S H_\alpha$ . Both may be defined briefly by the  $r \times r$  matrix  $c_j^i(S)$ , for which we have

$$\sum_{j=1}^r c_j^i(S) = \sum_{j=1}^r c_i^j(S) = \alpha_i, \quad i = 1, 2, \dots, r. \quad (4)$$

If a substitution  $S$  takes a digit  $j$  from a normal  $[\alpha]$ -tableau into the  $i$ th row, the inverse substitution  $S^{-1}$  will take a digit  $i$  into the  $j$ th row, so we have

$$c_j^i(S) = c_i^j(S^{-1}). \quad (5)$$

It will be convenient to use the symbol  $[\alpha]_\gamma$  to denote a tableau (or sequence) whose digits are taken from a normal  $[\gamma]$ -tableau, but are arranged in the form of a standard  $[\alpha]$ -tableau (or corresponding sequence), so that the digits are non-increasing in each of the rows of the latter. Thus, for example, let  $n = 7$ ,  $[\alpha] = [3, 2, 1, 1]$ ,  $[\gamma] = [5, 1, 1]$  and let  $S_1$  be the permutation  $(a)(bdecf)(g)$ . We chose  $S = H_\alpha S_1 H_\alpha = (ac) \cdot S_1 \cdot (acb)(de)(f)(g) = (af)(be)(c)(d)(g)$  so that  $[\alpha]^S$  is standard, and obtain the following tableaux:

NORMAL $[\alpha]$ ;	DERANGED $S_1[\alpha]$ ;	STANDARD $[\alpha]^S$ ;	$[\alpha]_\gamma^S$ ;	
111	132	321	111	
22	12	21	21	
3	1	1	1	(6a)
4	4	4	3	

and the corresponding sequences:

$$111, 22, 3, 4; \quad 132, 12, 1, 4; \quad 321, 21, 1, 4; \quad 111, 21, 1, 3. \quad (6b)$$

The method for transforming the  $[\alpha]^S$ -sequence to the  $[\alpha]_\gamma^S$ -sequence will be described in the next paragraph.

4. *Lattice Permutations and Sequences.*—A deranged sequence is said to be a lattice permutation of a normal sequence if among the first  $k$  digits,  $k = 1, 2, \dots, n$ , the frequency of any digit does not exceed the frequency of any lower digit. The procedure described by Robinson<sup>2</sup> and his prede-



cessors may be used to transform in a unique manner a standard  $[\alpha]$ -sequence into a lattice  $[\alpha]_\gamma$  sequence, i.e., into a sequence which is a lattice permutation of a normal  $[\gamma]$ -sequence but has the form of a standard  $[\alpha]$ -sequence. We may summarize their method as follows.

If we denote by  $m_{k,s}$  the number of times that the digit  $s$  occurs among the first  $k$  digits of the sequence, then if the sequence is non-lattice, some of the differences  $d_{k,s} = m_{k,s} - m_{k,s-1}$  must be positive. Pick the smallest  $s \geq 2$  for which some  $d_{k,s} > 0$ , then the largest difference  $d_{k,s}$  for that  $s$ , and then the smallest  $k$  for that difference. Change this  $k$ th digit from  $s$  to  $s - 1$ . If this change makes some  $d_{l,s-1} = 1$ , reapply the same rules to change the  $l$ th digit from  $s - 1$  to  $s - 2$ , etc., and suppose that finally when we change a  $t + 1$  to a  $t$  in this way that the digits less than  $s$  are again in lattice order. We now have a new tableau with  $\alpha_s - 1$  digits  $s$  and  $\alpha_t + 1$  digits  $t$ , and we associate with this reduction the operator  $C_{ts}$ . Starting all over again on the new tableau or sequence, if it is not already lattice, we make successive reductions until finally the standard  $[\alpha]$ -sequence is reduced to a lattice  $[\alpha]_\gamma$ -sequence. With this entire transformation we associate the operator

$$L_\lambda = \prod_{j=2}^r \prod_{i < j} C_{ij}^{\lambda_{ij}}, \quad (7)$$

where  $\lambda_{ts}$  denotes the number of digits  $s$  which are replaced by  $t$  according to the procedure just described. For  $t > s$  it is convenient to define  $\lambda_{ts} = -\lambda_{st}$ , so as to make the matrix  $(\lambda_{ij})$  skew-symmetric. The new partition  $[\gamma]$  is then given in terms of  $[\alpha]$  and  $\lambda$  by the equations

$$\gamma_i = \alpha_i + \sum_{j=1}^r \lambda_{ij}. \quad (8)$$

It may of course happen that the last  $r-r'$  digits  $\gamma_i$  may vanish. The inverse of the operator  $L_\lambda$  is defined by the transpose of the skew-symmetric matrix  $(\lambda_{ij})$ .

The number of solutions of (8) for given partitions  $[\alpha]$  and  $[\gamma]$  is known<sup>8</sup> to equal  $\mu_\gamma^\alpha$ , the number of times the irreducible representation  $\{\gamma\}$  corresponding to the partition  $[\gamma]$  occurs in the reduction of the permutation group  $G_H(\alpha)$ .

We are concerned in this proof with the reduction to lattice form of the standard  $[\alpha]$ -tableaux corresponding to an arbitrary but definitely chosen standard permutation  $S$  and its inverse  $S^{-1}$ . We shall denote these tableaux and the corresponding sequences by  $A$  and  $A'$ . We shall further distinguish between various operators  $C_{ts}$  by letting  $C_{ts,r} = C_t(s, s', s'', \dots, s^{(p)}; r, r', r'', \dots, r^{(p)})$  be an operator which starts by reducing a digit  $s$  in row  $r$  of the tableau, is thereby forced to reduce a digit in row  $r' \geq r$  whose original value was  $s' \leq s$ , then a digit in row  $r'' \geq r'$  whose original value

was  $s'' \leq s'$ , etc., and finally reduces to the value  $t$  a digit in row  $r^{(p)} \geq \dots r'' \geq r' \geq r$ , whose original value was  $s^{(p)} \geq \dots s'' \geq s' \geq s$ .

The following example will illustrate the reduction just described. Let  $n = 11$ , and  $[\alpha] = [3, 3, 2, 2, 1]$ . The permutations

$$S: (afeigkdj)(bc)(h) \quad \text{and} \quad S^{-1}: (ajdkgief)(bc)(h) \quad (9)$$

generate double cosets whose standard  $[\alpha]$ -tableaux  $A$ ,  $A'$ , and matrices  $c_j^i(S) = c_i^j(S^{-1})$  are the following:

$$\begin{array}{cc} \begin{array}{c} 411 \\ 521 \\ A: \quad 43 \\ 22 \\ 3 \end{array} & \begin{array}{c} 211 \\ 442 \\ A': \quad 53 \\ 31 \\ 2 \end{array} & c_j^i(S) = \begin{pmatrix} 2 & 0 & 0 & 1 & 0 \\ 1 & 1 & 0 & 0 & 1 \\ 0 & 0 & 1 & 1 & 0 \\ 0 & 2 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 \end{pmatrix}. \end{array} \quad (10)$$

The reduction of the corresponding sequences is as follows.

$$\begin{array}{ll} A: & 411, 521, 43, 22, 3 \text{ (standard)} \\ & -11, -21, --, 22, - \\ & -11, -21, -3, 22, 3 \\ C_{14,3} & -11, -21, \mathbf{32}, 21, 3 \\ C_{14,1} & 111, -21, 32, 21, 3 \\ A_\gamma: & C_{25,2} \quad 111, 221, 32, 21, 3 \text{ (lattice)} \\ & \underline{111, 222, 33, 44, 5} \text{ (normal)} \end{array} \quad \begin{array}{ll} A': & 211, 442, 53, 31, 2 \\ C_{12,1} & 111, --2, --, -1, 2 \\ C_{23,4} & 111, --2, -3, \mathbf{21}, 2 \\ C_{24,2} & 111, -22, -3, 21, 2 \\ C_{14,2} & 111, 222, -3, 11, 2 \\ A'_\gamma: & C_{35,3} \quad 111, 222, 33, 11, 2 \\ & \underline{111, 222, 33, 44, 5} \end{array} \quad (11)$$

$$L_\lambda = C_{14,3} C_{14,1} C_{25,2}, \quad L_{\lambda'} = C_{12,1} C_{23,4} C_{24,2} C_{14,2} C_{35,3}.$$

At each stage the digits  $\geq s$  are indicated by dashes, and the newly changed digits  $< s$  are shown in bold face type, with the corresponding operator  $C_{is,r}$  at the left. The final lattice sequences  $A_\gamma$  and  $A'_\gamma$  are followed by the normal  $[\alpha]$ -sequence for purposes of comparison. It is readily seen in this example (11) that the change from the normal  $[\alpha]$ -sequence to the lattice  $A_\gamma$  sequence is essentially given by the same operator  $L_\lambda$ , which takes  $A'$  into  $A'_\gamma$ , and that the change from the normal  $[\alpha]$ -sequence to  $A'_\gamma$  is given by  $L_\lambda$ , if a factor  $C_{is,r}$  is now interpreted to mean that a digit  $t$  appears in row  $s$ . The new significance for the subscript  $r$  is that when  $r \leq s$  there is a digit  $r$  in  $A$  which reduces to the digit  $t$  in row  $s$  of  $A$ , and corresponds to the operator  $C_{is,r}$  of  $L_\lambda$ . If  $r > s$ , however, and if there are  $f_s$  digits  $s$  in the tableau  $A_\gamma$ , which are in row  $s$ , then a final digit  $t < s$  in  $A$  occurs  $f_s$  places to the right of the corresponding  $r$  in  $A$ , in order that the standard form be preserved.

That this dual relationship of the operators  $L_\lambda$  and  $L_{\lambda'}$  is true in general will be shown in §5. In the special case considered by Robinson,<sup>2</sup> this was considered obvious. But in the more complicated situation treated in this paper, some indication of the proof seems to be required. From this

duality we see that the operators  $L_\lambda$  and  $L_{\lambda'}$  together determine the standard tableaux corresponding to both  $S$  and  $S^{-1}$ , their rôles being merely interchanged in the two cases. This is the crucial fact which will be used in §6 to establish our theorems.

5. *The Duality of the Operators for  $S$  and  $S^{-1}$ .*—We concentrate our attention on the effect of a factor  $C_{is,r}$  of  $L_{\lambda'}$  in the reduction of  $A'$ . At each stage in the reduction process, the need for reducing a digit  $s$  in row  $r$  is determined completely by the original arrangement of digits  $\leq s$  in rows  $< r$ , and other digits  $s$  which precede it in row  $r$ . Also a digit  $s$  in row  $r$  of  $A'$  corresponds to a certain digit  $r$  in row  $s$  of  $A$ , and we shall see that if the operator  $C_{is,r}$  is applied to this digit  $s$  in the reduction of  $A'$ , then the corresponding digit  $r$  in  $A$  will give rise to a digit  $t$  in the same row  $s$  of  $A$ . Let us first analyze the cases  $s = 2, 3$ . There are certain critical digits 2 in  $A'$  which reduce to 1. An operator  $C_{12,r}$  changing a critical digit 2 to 1 in row  $r > 2$  of  $A'$  will correspond to a change from  $r$  to 2 and 2 to 1 in row 2 of  $A$ , since at this stage in the reduction of  $A$  all digits  $< r$  in row 1 have become 1's, all the critical digits  $< r$  in row 2 have become 1's (as may be assumed by induction), and all other digits  $< r$  in row 2 have become 2's. Hence at the stage when this digit  $r$  is reduced, the 2's in row 2 just balance the 1's in row 1, and there are no digits between 2 and  $r - 1$  in row 1. Consequently this  $r$  must reduce to 2, and then the last 2 in the second row must reduce to 1.

The case  $s = 3$  is somewhat more typical. An operator  $C_{23,r}$  changing a 3 to 2 in row  $r \geq 2$  of  $A'$  will correspond in  $A$  to a change from  $r$  to 2 or from  $r$  to 3 and 3 to 2 in row 3. At this stage there are as many digits  $\leq r$  which have become 3's in row 3 as digits  $< r$  which have become 2's in row 2, but less digits  $< r$  which have become 2's in row 2 than digits  $< r$  which have become 1's in the first two rows. Furthermore, as the digits  $\geq r$  in the first three rows are successively reduced, at no stage will an excess of 2's be created in the third row which will force this 2 to become a 1. Hence each operator  $C_{23,r}$  for  $A'$  yields a 2 in row 3 of  $A$ . The operator  $C_{13,r}$  changing 3 to 1 will do so directly in  $A'$  if  $r = 1$ , but in rows  $r \geq 2$ , there may be a change from 3 to 2 in row  $r$  which causes a change from 2 to 1 in a different digit of some row  $r' \geq r$ . Then the original value of this new digit must have been  $s' \leq 3$ . To this situation in  $A'$  will correspond in  $A$  a digit  $r$  in row 3 which is reduced to 2, and a digit  $r' \geq r$  in row  $s' \leq 3$  which is subsequently reduced to 2, thereby causing an excess of 2's which forces a 2 in row 3 down to a 1. Hence each operator  $C_{13,r}$  belonging to  $A'$  yields a 1 in row 3 of  $A$ .

In general let the operator  $C_{is,r} = C_i(s, s', s'', \dots s^{(p)}; r, r', r'' \dots r^{(p)})$  be applied at the proper stage to the tableau  $A'$ . Then the reduction of the digit  $s$  in row  $r$  of  $A'$  may cause other digits further to the right in our sequence to be reduced in succession, until finally the last of them is reduced

to the value  $t$ . Let the original digits occupying these critical positions in  $A'$  be denoted by  $s', s'', \dots s^{(\phi)}$ , and let their rows be  $r', r'', \dots r^{(\phi)}$ . Then as stated in §4 the values of  $r^{(k)}$  are non-decreasing, while those of  $s^{(k)}$  are non-increasing. The first statement is obvious from the rules of lattice reduction; the second statement, though less obvious can be verified by studying the preceding stages at which the digits  $s^{(k)}$  were reduced. If  $s > r$ , then our original digit  $s$  must be reduced directly at least as far as  $r$ , since all digits between  $r$  and  $s - 1$  inclusive have already been eliminated from the first  $r - 1$  rows. Now our critical digits  $s^{(k)}$  in row  $r^{(k)}$  of  $A'$  correspond to certain digits  $r^{(k)}$  in row  $s^{(k)}$  of  $A$ . We select a subsequence  $(r_k, s_k)$  of the pairs  $r^{(k)}, s^{(k)}$  so as to eliminate equalities among the digits  $s^{(k)}$ , and also to eliminate equalities among the  $r^{(k)}$  when  $r^{(k)} < s^{(k)}$ . If  $s > r$ , we reject  $s$  itself when  $r = t$  or  $r < r'$ , but set  $s = s_1$  when  $r = r' > t$ , and choose  $s_2$  so that  $r < r_2$ . If  $s \leq r$ , we set  $s = s_1$ . If several of the  $s^{(k)}$  are equal, we reject all but the one corresponding to the largest  $r^{(k)}$ . If  $s^{(k)} > r^{(k)}$  and several  $r^{(k)}$  are equal, we reject all but the largest of these  $s^{(k)}$ . We denote the resulting monotone decreasing subsequence of the  $s^{(k)}$  by  $s_1 > s_2 > \dots s_q > t$ , and the corresponding values of the  $r^{(k)}$  by  $r_1 \leq r_2 \leq \dots \leq r_q$ . These digits mark certain steps in the reduction of  $A'$  which correspond to the successive steps in the reduction of the digit  $r$  of row  $s$  in  $A$ . We find that  $q$  is the smaller of the two numbers  $s - t$  and  $r - t$ . If  $s > r$ , then the  $r - t$  reductions in the digit  $r$  of row  $s$  in  $A$  are caused successively by the reductions in the digits  $r_1 \dots r_q$  in the previous rows  $s_1 \dots s_q$ . If  $r > s$ , then the digit  $r$  of row  $s$  in  $A$  is reduced directly to  $s$ , and the  $s - t$  further reductions which take place either directly in this digit or in some other digit further to the left in the same row are caused by reductions in the digits  $r_1 \dots r_q$  which successively upset the lattice arrangement in digits less than  $s$ .

Thus we see that the operator  $C_{ts, r}$  in the reduction of  $A'$  has as its counterpart in the reduction of  $A$  a succession of operations which change an  $r$  in row  $s$  of  $A$  to a  $t$  in row  $s$  of  $A_\gamma$ . Hence the tableau  $A$  which corresponds to the double coset generated by  $S$  is defined by the operators  $L_\lambda$ , and  $L_\lambda$ , and in like manner the tableau  $A'$  corresponding to the inverse double coset is defined by these same operators taken in reverse order.

6. *Conclusion.*—In view of this duality between the operators  $L_\lambda$  and  $L_{\lambda'}$ , we may arrange all the double cosets  $H_\alpha S H_\alpha$  whose standard  $[\alpha]$ -tableaux reduce to  $[\gamma]$ -tableaux belonging to the same partition  $[\gamma]$  in a square array whose rows and columns may be designated by  $\lambda$  and  $\lambda'$ , respectively. Then since we have  $\lambda = \lambda'$  in the main diagonal, the diagonal double cosets will be self inverse, whereas in general the pairs of inverse double cosets will be symmetrically placed with respect to the diagonal. For each partition  $[\gamma]$  we have as many rows in the square matrix of double cosets as there are operators  $L_\lambda$ , namely  $\mu_\gamma^\alpha$ . Consequently there are

$(\mu_\gamma^\alpha)^2$  double cosets associated with the partition  $[\gamma]$  of  $n$ . Since the total number of double cosets  $H_\alpha S H_\alpha$  is  $\sum_\gamma (\mu_\gamma^\alpha)^2$ , we have just one square of double cosets for each partition  $[\gamma]$  which corresponds to an irreducible component  $\{\gamma\}$  in the reduction of the permutation group  $G_H(\alpha)$ . If in this array we examine the placement of inverse double cosets, we see that Theorem I follows at once. If we count the number of self-inverse double cosets, we see that their number is  $\sum_\gamma \mu_\gamma^\alpha$ , and thus we prove Theorem II.

<sup>1</sup> Littlewood, D. E., and Richardson, A. R., *Phil. Trans. Roy. Soc. London (A)* **233**, 99-141 (1934).

<sup>2</sup> Robinson, G. deB., "On the Representations of the Symmetric Group," *Amer. Jour. Math.*, **60**, 745-759 (1938).

<sup>3</sup> Murnaghan, F. D., "The Theory of Group Representations," Johns Hopkins Press, Baltimore (1938), esp. pp. 150 ff.

## GENERAL EXPANSION THEOREMS

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A set  $G$  of elements of a normed linear space<sup>1</sup>  $E$  is said to be fundamental if every element of  $E$  is the limit of a sequence of finite linear combinations of elements of  $G$ ; it is called a base if every element  $x$  of  $E$  can be expressed uniquely in the form

$$x = \sum_{n=0}^{\infty} a_n y_n, \quad y_n \in G. \quad (1)$$

Theorems of G. D. Birkhoff,<sup>2, 3</sup> J. L. Walsh<sup>4</sup> and R. E. A. C. Paley and N. Wiener<sup>5</sup> illustrate the general principle that if the elements of two sets  $G_1$  and  $G_2$  are sufficiently near each other, in some suitable sense, then either set is fundamental, or is a base, if the other is. More precisely, the following theorem can be proved.

**THEOREM 1.** Let  $\{x_n\}$  and  $\{y_n\}$  be two sequences of elements of the normed linear space  $E$ . If there is a number  $\lambda$ ,  $0 < \lambda < 1$ , such that for every sequence  $\{a_n\}$  of complex numbers

$$\left\| \sum_{n=1}^N a_n (x_n - y_n) \right\| \leq \lambda \left\| \sum_{n=1}^N a_n x_n \right\|, \quad N = 1, 2, \dots, \quad (2)$$

then if  $\{x_n\}$  is a fundamental set or a base,  $\{y_n\}$  has the same property



Theorem 1 for bases can be proved by a generalization of the proof given by Paley and Wiener when  $E$  is Hilbert space; a proof of Theorem 1 for fundamental sets is much simpler, and this weaker result suffices for many applications.

To show how Theorem 1 can be applied, I shall outline proofs of some theorems on the expansion of analytic functions of a complex variable in terms of functions  $z^n[1 + h_n(z)]$ , where the functions  $h_n(z)$  are "small." These theorems include and generalize a number of expansions which occur in the literature; in some cases, the methods of this note lead to larger regions of validity for the expansions than have been obtained previously. One special case can be applied to a conjecture concerning the zeros of the derivatives of an entire function of exponential type.

For the space  $E$ , I take the set of functions  $f(z)$ , analytic in  $|z| < r$  and continuous in  $|z| \leq r$ , with the norm

$$\|f\| = \left\{ \frac{1}{2\pi} \int_0^{2\pi} |f(re^{i\theta})|^p d\theta \right\}^{1/p}, \quad p \geq 1 \quad (3)$$

$G_1$  is the set  $\{z^n\}$ ,  $n = 0, 1, 2, \dots$ ;  $G_2$  is a sequence of elements  $\{g_n(z)\}$  of  $E$ . Then Theorem 1 yields

**THEOREM 2.** *If, for every sequence  $\{a_n\}$  of complex numbers, some number  $p$ ,  $p \geq 1$ , and every  $N$ ,*

$$\int_0^{2\pi} \left| \sum_{n=0}^N a_n [z^n - g_n(z)] \right|^p d\theta \leq \lambda^p \int_0^{2\pi} \left| \sum_{n=0}^N a_n z^n \right|^p d\theta, \quad (4)$$

where  $z = re^{i\theta}$ ,  $\lambda < 1$ , then any function  $f(z)$ , analytic in  $|z| < r$  and continuous in  $|z| \leq r$ , can be expanded in a series of the functions  $g_n(z)$ , the series converging uniformly in any circle  $|z| \leq r' < r$ .

The direct deduction from Theorem 1 is that there exist coefficients  $c_n$  such that

$$\lim_{n \rightarrow \infty} \int_0^{2\pi} |f(re^{i\theta}) - \sum_{k=0}^n c_k g_k(re^{i\theta})|^p d\theta = 0. \quad (5)$$

For  $z = r'e^{i\theta}$ ,  $r' < r$ , one can represent

$$f(r'e^{i\theta}) - \sum_{k=0}^n c_k g_k(r'e^{i\theta})$$

by Cauchy's integral along  $|z| = r$ , and apply Hölder's inequality to deduce the conclusion of Theorem 2 from (5).

Birkhoff's expansion theorem<sup>3</sup> can be deduced in a few lines from Theorem 2, with  $1 < p \leq 2$ . Another theorem which can be obtained from



Theorem 2 resembles a theorem of S. Takenaka,<sup>6</sup> which has been shown<sup>7</sup> to contain a large number of special expansion theorems.

**THEOREM 3.** *If the functions  $h_n(z)$  are analytic in  $|z| < r_0$ , vanish at  $z = 0$ , and have a common majorant  $h(z)$  satisfying*

$$\frac{1}{2\pi} \int_0^{2\pi} |h(\rho e^{i\theta})|^2 d\theta \leq K_\rho^2, \quad 0 < \rho < r_0; \quad (6)$$

*then any function  $f(z)$ , analytic in  $|z| < s$ , where  $s \leq r_0$ , can be expanded in a (uniformly convergent) series of the functions  $z^n [1 + h_n(z)]$  in any circle*

$$|z| \leq s_1 < \min \left\{ s, \sup_{0 < \rho < r_0} \rho (K_\rho^2 + 1)^{-1/2} \right\}.$$

With  $p = 2$ , and  $g_n(z) = 1 + h_n(z)$ , (4) becomes

$$\frac{1}{2\pi} \int_0^{2\pi} \left| \sum_{n=0}^N a_n r^n e^{in\theta} h_n(re^{i\theta}) \right|^2 d\theta \leq \lambda^2 \sum_{n=0}^N |a_n|^2 r^{2n}, \quad (7)$$

where  $s_1 < r < r_0$ . Let

$$\sum_{n=0}^N |a_n| z^n = \sum_{n=0}^{\infty} |a'_n| z^n = \varphi(z), \quad a'_n = 0 (n > N);$$

$$h_n(z) = \sum_{k=1}^{\infty} \gamma_k^{(n)} z^k, \quad h(z) = \sum_{k=1}^{\infty} \gamma_k z^k,$$

where  $|\gamma_k^{(n)}| \leq \gamma_k$  for  $k = 1, 2, \dots; n = 0, 1, 2, \dots$

The left side of (7) is easily reduced to the form

$$\sum_{m=1}^{\infty} r^{2m} \left| \sum_{n=0}^{m-1} a'_n \gamma_{m-n}^{(n)} \right|^2. \quad (8)$$

The expression inside the absolute value signs does not exceed in absolute value the coefficient of  $z^m$  in the power series of  $\varphi(z)h(z)$ , and consequently does not exceed

$$\left| \frac{1}{2\pi i} \int_{|z|=\rho} \frac{h(z)\varphi(z)}{z^{m+1}} dz \right|, \quad r < \rho < r_0;$$

and the square of its absolute value does not exceed

$$\frac{K_\rho^2}{2\pi \rho^{2m}} \int_0^{2\pi} |\varphi(\rho e^{i\theta})|^2 d\theta = \frac{K_\rho^2}{\rho^{2m}} \sum_{n=0}^{m-1} |a'_n|^2 \rho^{2n}.$$

Substitution in (8), followed by some algebraic reductions, yields

$$\frac{K_\rho^2 r^2}{\rho^2 - r^2} \sum_{n=0}^N |a_n|^2 r^{2n}$$

as an upper bound for (8), and thus (7) is true with  $\lambda < 1$  if  $\rho$  is chosen so that

$$r < \rho(K_\rho^2 + 1)^{-1/2}.$$

A particular set of functions coming under the hypotheses of Theorem 3 is the set  $\{z^n e^{\alpha_n z}\}$ , where the  $\alpha_n$  are complex numbers such that  $|\alpha_n| \leq 1$ . These functions are of interest because, if every function analytic in the circle  $|z| < R$  can be expanded in a series of them, the following theorem can be deduced.

THEOREM 4. *If  $F(z)$  is an entire function such that<sup>1</sup>*

$$\limsup_{r \rightarrow \infty} \frac{1}{r} \log M(r) < R,$$

*and  $F(z)$  and each of its derivatives have a zero inside or on the unit circle, then  $F(z) \equiv 0$ .*

Theorem 4 has been established by Takenaka<sup>2</sup> with  $R = \log 2 = 0.693+$ . It has been conjectured<sup>3</sup> that it is true with  $R = \pi/4 = 0.785+$  [which would be "best possible:" example,  $F(z) = \sin(\pi z/4) - \cos(\pi z/4)$ ]; the conjecture has been verified by I. J. Schoenberg<sup>10</sup> when all the zeros are required to lie on the real axis.

For the functions  $h_n(z) = z^n(e^{\alpha_n z} - 1)$ , (8) does not exceed

$$\sum_{m=1}^{\infty} r^{2m} \left\{ \sum_{n=0}^{m-1} \frac{|a'_n|}{(m-n)!} \right\}^2;$$

by retracing the steps which led to (8), one sees that the left side of (7) does not exceed

$$\frac{1}{2\pi} \int_0^{2\pi} \left| (e^s - 1) \sum_{n=0}^N |a_n| r^n e^{in\theta} \right|^2 d\theta.$$

This yields at once the expansion theorem with  $R = \log 2$  (it was known previously, I believe, only for  $R = 1/e$ ), and so establishes Takenaka's result for Theorem 4.<sup>11</sup> It seems likely that still more careful estimates will yield the value  $R = \pi/4$ .

Detailed proofs, with extensions of the special theorems and other applications of Theorem 1, will be published elsewhere.

<sup>1</sup> The terminology is that of S. Banach's book, *Théorie des opérations linéaires*, 1932, except that Banach's real linear spaces are replaced by complex linear spaces.

<sup>2</sup> These PROCEEDINGS, 3, 656-659 (1917).

<sup>3</sup> C. R. Acad. Sci. Paris, 163, 942-945 (1917).

<sup>4</sup> Trans. Amer. Math. Soc., 31, 53-57 (1929). Other references will be found in this paper.

<sup>5</sup> Fourier Transforms in the Complex Domain, 100 (1934).

<sup>6</sup> *Proc. Phys.-Math. Soc. Japan* (3), 13, 111-132 (1931).

<sup>7</sup> See G. S. Ketchum, *Trans. Amer. Math. Soc.*, 40, 208-224 (1936), where further references are given.

<sup>8</sup>  $M(r)$  denotes the maximum of  $|F(z)|$  for  $|z| = r$ .

<sup>9</sup> See J. M. Whittaker, *Interpolatory Function Theory*, 44-45 (1935).

<sup>10</sup> *Trans. Amer. Math. Soc.*, 40, 12-23 (1936).

<sup>11</sup> The method indicated here can be developed further: if (6) of Theorem 3 is replaced by  $|h(re^{i\theta})| \leq K_r$ , the expansion can be shown to be possible in any subcircle of  $|z| < s$  in which  $K_r < 1$ ; Takenaka's theorem can easily be deduced from this result.

## SOME COMBINATORIAL PROPERTIES OF COMPLEXES

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1. *Introduction.*—The purpose of this note is twofold. First, we give a definition of the "dual" of a cell in a larger cell, and use this to define products in a complex.<sup>1</sup> Second, we discuss "locally isomorphic" complexes, and products in such complexes. The results are needed in the following note.

2. *Dual Systems in a Complex.*—For each pair of oriented cells  $\sigma^r, \sigma^{r+s}$  in a geometric complex  $K$ , let

$$D(\sigma^r, \sigma^{r+s}) = \text{dual of } \sigma^r \text{ in } \sigma^{r+s}$$

be a singular  $s$ -chain whose cells are in  $\sigma^{r+s}$ , and each cell of the closure of which is in either  $\sigma^{r+s}$  or  $\sigma^r$ . Let it be 0 if  $\sigma^r$  and  $\sigma^{r+s}$  are not incident. Set

$$D(\sum \alpha_i \sigma_i^r, \sum \beta_j \sigma_j^{r+s}) = \sum \alpha_i \beta_j D(\sigma_i^r, \sigma_j^{r+s}).$$

If these satisfy the two conditions

$$D(x_i, x_i) = x_i \quad (\text{for each vertex } x_i),$$

$$\partial D(\sigma^r, \sigma^{r+s}) = (-1)^{r+1} [D(\delta \sigma^r, \sigma^{r+s}) - D(\sigma^r, \partial \sigma^{r+s})],$$

we say these form a system dual to  $K$ .

3. *Intersections in a Cell.*—Let  $E'^n$  be an oriented half-plane bounded by  $E^{n-1}$ :  $\partial E'^n = E^{n-1}$ . Let

$$\sigma^r = x_{\lambda_1} \dots x_{\lambda_r}, \sigma^s = x_{\mu_1} \dots x_{\mu_s}, (r + s = n),$$

be simplexes in  $E'^n + E^{n-1}$ . With a simple definition of *general position relative to  $E^{n-1}$* , we may define their Kronecker index  $\{\sigma^r, \sigma^s, E'^n\}$  as usual;

we omit anything in  $E^{n-1}$ . (The cells may be singular, if any one is wholly in  $E'^n$  or  $E^{n-1}$ .) Let their index in any  $E^*$  be 0 if they are not both in  $E^*$ . Then

$$\{A', \partial B^{r+s}, E'^n\} - (-1)^r \{\partial A', B^s, E'^n\} + \{\partial A', \partial B^s, \partial E'^n\} = 0.$$

Applying the same theory to a cell and its faces gives the same formula with  $E'^n$  replaced by a cell or chain  $C^n$ .

4. *Products in Terms of Intersections.*—Say two systems  $D, D'$  dual to  $K$  are in *general position* if their sets of singular cells are in general position in each  $\sigma'$  relative to  $\partial\sigma'$ .

If this holds, and we set

$$(\sigma' \smile \sigma^s) \cdot \sigma^{r+s} = \{D(\sigma', \sigma^{r+s}), D'(\sigma^s, \sigma^{r+s}), \sigma^{r+s}\},$$

then the  $\smile$  and corresponding  $\frown$  products satisfy the conditions in PC.

5. *Special Products.*—The products of PC, §6, may be obtained as follows: Let  $K$  be simplicial, with ordered vertices. Take  $0 < a < 1$ , and set

$$y_{\lambda_0} = x_{\lambda_0}, \quad y_{\lambda_0 \dots \lambda_r} = (1-a)x_{\lambda_0} + ay_{\lambda_1 \dots \lambda_r} \quad (\lambda_0 < \dots < \lambda_r).$$

$y_{\lambda_0 \dots \lambda_r}$  is interior to  $x_{\lambda_0} \dots x_{\lambda_r}$ . Let

$$[\lambda'_0 \dots \lambda'_r] = +1 \text{ or } -1$$

according as  $(\lambda'_0, \dots, \lambda'_r)$  is an even or odd permutation of the natural order of these numbers. If  $(\lambda'_0, \dots, \lambda'_r)$  is a permutation of the natural order  $\lambda_0 < \dots < \lambda_r$ , we use

$$x_{\lambda_0} \dots x_{\lambda_r} = [\lambda'_0 \dots \lambda'_r] x_{\lambda'_0} \dots x_{\lambda'_r}, \quad y_{\lambda'_0} \dots y_{\lambda'_r} = y_{\lambda_0 \dots \lambda_r}.$$

Now take any cells

$$\sigma' = x_{\lambda_0} \dots x_{\lambda_r}, \quad \sigma^{r+s} = [\lambda_0 \dots \lambda_r \dots \lambda_{r+s}] x_{\lambda_0} \dots x_{\lambda_r} \dots x_{\lambda_{r+s}}, \quad \lambda_0 < \dots < \lambda_r, \\ \lambda_{r+1} < \dots < \lambda_{r+s},$$

and set

$$D_a(\sigma', \sigma^{r+s}) = \sum_{(\mu)} [\lambda_0 \dots \lambda_r \mu_1 \dots \mu_s] y_{\lambda_0 \dots \lambda_r} y_{\lambda_0 \dots \lambda_r \mu_1} \dots y_{\lambda_0 \dots \lambda_r \mu_1 \dots \mu_s},$$

summed over all permutations  $(\mu_1, \dots, \mu_s)$  of  $(\lambda_{r+1}, \dots, \lambda_{r+s})$ . Then *this is a system dual to  $K$* . If  $0 < a' < a < 1$ , then the systems  $D_a, D_{a'}$  are in *general position*, and the definition in §4 gives the products of PC, §6. (This is easy to verify for  $r+s \leq 2$ .) If we replace  $a'$  by 0 and  $a$  by 1, we find interesting combinatorial formulas.

6. *A New Particular  $\smile$  Product.*—Writing  $A^r = \sum a_{\lambda_0 \dots \lambda_r} x_{\lambda_0} \dots x_{\lambda_r}$ , etc., then  $A^r \smile B^s = C^{r+s}$  may be defined as follows, for various  $r$  and  $s$ :

$$c_{\lambda} = a_{\lambda} b_{\lambda}, \quad c_{\lambda_0 \dots \lambda_r} = a_{\lambda_0 \dots \lambda_r} b_{\lambda_0}, \quad c_{\lambda_0 \dots \lambda_s} = a_{\lambda_1} b_{\lambda_0 \lambda_1 \dots \lambda_s}, \quad c_{\lambda_0 \dots \lambda_r + s} = (-1)^{r+s+1} a_{\lambda_1 \dots \lambda_{r+1}} (b_{\lambda_1 \lambda_{r+1} + 1 \dots \lambda_r + s} - b_{\lambda_0 \lambda_{r+1} + 1 \dots \lambda_r + s}).$$

This product is used in the following note, §6.

7. *Locally Isomorphic Complexes.*—Let  $K$  be an abstract complex. In place of  $(K_4)$  of PC, we assume: (a) Each  $\sigma^1$  is incident with exactly two vertices;  $\partial\sigma^1 = \pm a \pm b$ . (b) Each  $\partial\sigma^r$  ( $r > 1$ ) is combinatorially connected (through paths with non-zero incidence numbers). (c) 0- and 1-cycles mod 2 in any closed cell  $\bar{\sigma}$  bound in  $\bar{\sigma}$ , if the 0-cycles have an even number of vertices. Two complexes  $K, K'$  are *locally isomorphic* if there is a (1-1) correspondence  $\phi$  between their non-oriented cells which preserves incidences, and such that for each  $\sigma, \bar{\sigma}$  and  $\phi\bar{\sigma}$  are isomorphic complexes. Examples:

$$\begin{aligned} K: \quad \partial(ab) &= b - a, \quad \partial(ac) = c - a, \quad \partial(bc) = c - b, \\ K': \quad \partial'(ab) &= b - a, \quad \partial'(ac) = c - a, \quad \partial'(bc) = -c - b. \end{aligned}$$

If  $\sigma = bc$ , then reorienting  $c$  to  $c'$  in  $K'$  gives  $\partial'(bc') = \partial(bc)$ .  $K'$  has 1-dimensional torsion.

Note that chains mod 2 may be transferred to locally isomorphic complexes at will.

A *unit 0-chain*  $I$  is a sum  $\sum (\pm x_i)$  of all oriented vertices. Let  $a_2$  be the integer  $a$  mod 2; let  $A|_2 = (A)_2$  be the chain  $A$  reduced mod 2.

For any  $I$ ,  $W = (\frac{1}{2}\delta I)_2$  exists and is a cocycle; its cohomology class  $W$  is independent of the  $I$  chosen. If  $K$  and  $K'$  are locally isomorphic, then they are isomorphic if and only if  $W = W'$ .

$K$  is *normal* if  $W = 0$ .<sup>2</sup> Any geometric complex is normal. Given any normal  $K$  and any 1-cocycle  $X$  mod 2 in  $K$ , there is a locally equivalent  $K'$  with  $W' = X$ .

8. *Particular Orientations in Simplicial Complexes.*—Set

$$\rho(0_2) = 1, \quad \rho(1_2) = -1, \quad \rho(\sum \alpha_i \sigma_i) = \sum \rho(\alpha_i) \sigma_i.$$

Let  $\sigma_i$  denote oriented cells in either of the locally isomorphic complexes  $K, K'$ ,  $K$  being normal; the incidence numbers may differ in sign. Define new incidences in  $K'$  as follows. If  $\sigma^r, \sigma^{r+1}$  have the same first vertex, let  $[\sigma^r: \sigma^{r+1}]' = [\sigma^r: \sigma^{r+1}]$ . If their first vertices  $x_i, x_j$  are different, set

$$[\sigma^r: \sigma^{r+1}]' = \rho(W' \cdot (x_i x_j)) [\sigma^r: \sigma^{r+1}].$$

The  $K'$  thus constructed is isomorphic with the given one. (The cells may be reoriented.) Thus, in the example, we may use  $W' = (bc)_2$ ,  $\rho(W' \cdot bc) =$

$\rho(1_2) = -1$ , and  $[c:bc]' = -[c:bc] = -1$ . We may also write, with the  $\frown$  product of PC, §6,

$$[\sigma':\sigma'+1] = \rho[W' \cdot (\sigma' \frown [\sigma'+1])][\sigma':\sigma'+1].$$

9. *Products in General Complexes.*—The theory in PC holds only in normal complexes. It may be extended as follows. Let  $K', K'', K'''$  be locally isomorphic complexes, with

$$W' + W'' + W''' = 0.$$

Then we may define products of the form

$$X' \smile Y'' = Z''', \quad X'' \frown A''' = B',$$

as follows. Orient the vertices  $x'_i$ , etc., so that if  $I' = \Sigma x'_i$ , etc., then

$$\left(\frac{1}{2}\delta I'\right)_2 + \left(\frac{1}{2}\delta I''\right)_2 + \left(\frac{1}{2}\delta I'''\right)_2 = 0.$$

Let  $K$  be the corresponding normal complex, in which  $\frown_0$  is defined. Take any face  $\sigma_1$  of any  $\sigma_2$ ; choose a vertex  $x$  in  $\sigma_1$ . Let  $\Phi'$ , etc., be isomorphisms of  $\bar{\sigma}_2$  into  $\bar{\sigma}'_2$ , etc., such that  $\Phi'(x) = x'$ , etc. Then set

$$\sigma'_1 \frown \sigma'_2 = \Phi'[(\Phi'')^{-1}(\sigma''_1) \frown_0 (\Phi''')^{-1}(\sigma'''_2)].$$

If  $K$  is simplicial, with ordered vertices, we may use, if  $\lambda_0 < \dots < \lambda_p < \dots < \lambda_p + q$ ,

$$(x_{\lambda_p} \dots x_{\lambda_p+q})'' \frown (x_{\lambda_0} \dots x_{\lambda_p} \dots x_{\lambda_p+q})''' = \rho[W''(x_{\lambda_0} x_{\lambda_p})](x_{\lambda_0} \dots x_{\lambda_p})'.$$

The products are unique just as in PC.

10. *Subdivisions; Chain-Mappings.*—Any complex  $K'$ , in which each  $\bar{\sigma}$  has the homology groups of a closed simplex, is locally isomorphic with a similar normal complex  $K$ ; the latter has a "regular" subdivision, with geometric meaning. The theory of chain-mappings<sup>3</sup> carries over to the present case. Products are preserved under subdivision and chain-mappings. If  $K'_1$  is the "regular" subdivision of  $K'$ , then  $W' = Sd'W'_1$ .

11. *Coboundary Operators.*—Set  $\omega(0_2) = 0$ ,  $\omega(1_2) = -1$  (we could use  $+1$ ). Set  $\omega(\Sigma \alpha_i \sigma'_i) = \Sigma \omega(\alpha_i) \sigma'_i$  ( $\alpha_i = 0_2$  or  $1_2$ ). Then for any  $p$ - $I_2$ -cocycle  $X$ ,  $\frac{1}{2}\delta\omega X$  is a  $(p+1)$ - $I_0$ -cocycle whose cohomology class depends only on that of  $X$ . The dual operation  $\frac{1}{2}\partial\omega A$  is well known.

If  $K'$  and  $K''$  are locally isomorphic, and we let  $\sigma'_i$  denote cells in either, then  $\frac{1}{2}(\delta''A' - \delta'A')$  is defined for all chains  $A'$  in  $K'$ ; as a chain in  $K''$ , if  $\delta'A' = 0$ , it is a cocycle whose class depends only on that of  $A'$ . If  $\delta'A' = 0$ , we may write it as  $\frac{1}{2}\delta''\omega(A|_2)$ .



Corresponding to the pair  $K', K''$  is a characteristic  $2-I_0$ -cocycle in the corresponding complex  $K'''$ ,

$$X''' = \frac{1}{4}\delta''\delta'I' \quad (I' = \sum x'_i).$$

If  $\mathfrak{B}', \mathfrak{B}''$  are 0-sphere-bundles with these as classes, then their product has the 2-class  $X'''$  (see the following note). Similarly, to  $K', K'', K'''$  corresponds  $\frac{1}{8}\delta'''\delta''\delta'I'$ , etc.

12. *Relation to Products.*—Using  $K', K'', K'''$  again, and  $\omega A = A$  if  $A$  has integer coefficients, set

$$X' \cup Y'' = \omega X' \cup \omega Y''.$$

This has topological significance if each chain either is integral or equals  $W'$  or  $W''$ . To show this, we use, if  $\sigma_2$  is a  $p$ -face of  $\sigma_1 = \sigma_1^p + 1$ , and  $x_2, x_1$  are their first vertices,

$$\frac{1}{2}(\delta'''\sigma_2 - \delta''\sigma_2) \cdot \sigma_1 = \rho[W''(x_1x_2)]\omega W'(x_1x_2).$$

We find

$$W' \cup Y'' = \frac{1}{2}(\delta'''\omega Y'' - \delta''\omega Y''),$$

$$W' \cup W'' = \frac{1}{2}(\delta'''\omega W'' - \delta''\omega W'') = \text{the } X''' \text{ above.}$$

13. *On Non-Orientable Manifolds.*<sup>1</sup>—Let  $K$  be a subdivision of the non-orientable manifold  $M$ . For each oriented  $\sigma$ , let  $\epsilon$  be an orientation of the part of  $M$  about  $\sigma$ ; set

$$\sigma' = (\sigma, \epsilon) = (-\sigma, -\epsilon), \quad -\sigma' = (-\sigma, \epsilon) = (\sigma, -\epsilon), \quad [\sigma'_1: \sigma'_2] = [\sigma_1: \sigma_2] \text{ or } -[\sigma_1: \sigma_2]$$

according as the orientations  $\epsilon_1$  and  $\epsilon_2$  agree or disagree. The new complex  $K'$  is locally isomorphic with  $K$ ; its class has the property that any 1-circuit  $A^1$  in  $K$  preserves orientation if and only if  $A^1 \cdot W' = 0_2$ . The Poincaré duality theorem (compare PC, §18) says:

$${}^p\mathbf{H}_G(K) \approx {}^{n-p}\mathbf{H}^G(K'), \quad {}^p\mathbf{H}_G(K') \approx {}^{n-p}\mathbf{H}^G(K).$$

Note that in  $K'$ , the sum of the  $n$ -cells forms a cycle with integral coefficients.

<sup>1</sup> We recall the notations  $\partial$  for boundary,  $\delta$  for coboundary;  $I_\mu =$  integers mod  $\mu$ ,  $I_0 =$  integers. An  $r$ - $I_\mu$ -chain is an  $r$ -chain with coefficients in  $I_\mu$ . The reference PC will mean Whitney, "On Products in a Complex," *Ann. Math.*, 39, 397-432 (1938). We note some corrections to this paper: In footnote 9, 3-sphere should read 3-complex.

In Theorem 12, we mean the (uniquely determined) products of 11 (a): In place of the following paragraphs, read: As knowing all  $v_i^p \cdot u^p$  for all  $i$  uniquely determines  $u^p$ , and the correct  $\sim$  satisfies (11.8), we have found the correct  $\sim$ . (NOTE: Theorem 11 is not used.) The end of the first paragraph in 12 should read: Find  $u^p \sim u^q$  over  $I_0$ , then over  $R_1$ , ( $p \leq 2$ ), then for all  $p$ , by (5.12); then find  $\sim$  over  $I_0$  by Theorem 12. (We must know (11.15).) In Theorem 13, (a), add:  $\phi\tau^p = 0$  if  $O(\tau^p)$  is acyclic. Relation (14.9) follows directly from (14.4). After (25.7), add:  $I^p \sim I^q = I^{p+q}$ .

<sup>2</sup> *Augmentable* in Tucker, *Ann. Math.*, **34**, 191–243 (1933).

<sup>3</sup> See Tucker, these PROCEEDINGS, **25**, 371–374 (July, 1939). The theory was developed independently by S. Lefschetz and myself.

<sup>4</sup> This case of the theory is due to de Rham; see *Comm. Math. Helv.*, **4**, 151–157 (1933).

## ON THE THEORY OF SPHERE-BUNDLES

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1. *Introduction.*—We give here a brief sketch of some new results in the theory of sphere-bundles;<sup>1</sup> in particular, further properties of the characteristic classes, a duality theorem, theorems on tangent and normal bundles to a manifold, and some examples. The results will be published later in book form.

2. *Fibre-Bundles.*—Let  $S_0$  be a space, and  $G$ , a group of homeomorphisms of  $S_0$  into itself. Then over any space  $K$ , the *base space*, with neighborhoods  $U_i$ , we may define a *fibre-bundle*  $\mathfrak{B}(K)$  as follows. For  $p \in U_i$ , let  $\xi_i(p)$  be a homeomorphism of  $S_0$  into a set of points  $S(p)$ . Let  $S(p) \cdot S(q) = 0$  if  $p \neq q$ . Let  $\mathfrak{S}(K)$  be the space of all points on all  $S(p)$ , the *total space* (gefaserte Raum). Let  $\xi_i(p, q)$  be the image of  $q$  in  $S_0$  under  $\xi_i(p)$ . For  $p \in U_i \cdot U_j$ , set  $\xi_{ij}(p, q) = \xi_i^{-1}(p, \xi_j(p, q))$ ; assume  $\xi_{ij}(p) \in G$  for each  $p$ , and that it varies continuously with  $p$ . Then a topology is easily defined in  $\mathfrak{S}$ . The part  $\mathfrak{S}(U_i)$  of  $\mathfrak{S}$  over  $U_i$  is a product  $U_i \times S_0$ .

If  $S_0$  is a set of  $\mu$  points, and  $G$  is the group of permutations, we obtain the covering spaces of  $K$  with  $\mu$  sheets. If  $S_0$  is a subgroup of a continuous group  $R_0$ , and  $G = S_0$ , then the left (or right) cosets of  $S_0$  form a space  $K$  (factor group if  $S_0$  is normal); the total space is  $R_0$ . If  $S_0 = S_0'$  is a  $\nu$ -sphere, and  $G = G'^{+1}$ , the orthogonal group, we have a *sphere-bundle*. If  $S_0$  is a vector space, and  $G$ , the affine or orthogonal group, an equivalent theory is obtained.

3. *Particular Coördinate Systems.*—We use this section in the proof of the duality theorem. Let  $K$  be a complex with ordered vertices. We may use  $\xi_\sigma$ , defined over closed cells  $\sigma$ . (See TP.) We may choose them so  $\xi_\sigma = \xi_{\sigma'}$  if  $\sigma$  and  $\sigma'$  have the same first vertex. Let  $P'$  be a small closed

region in  $K$  surrounding all  $D_\sigma(\sigma', \sigma')$ ,  $\sigma'$  of any dimension (see I, §2); set  $Q' = K - P'$ . Then if  $K'$  is the  $r$ -dimensional part of  $K$ , and  $e_1, \dots, e_{\nu+1}$  are the unit points of  $S_0''$ ,

$$\xi_{\sigma, \sigma'}(p, e_i) = e_i \quad (p \in Q^k \cdot \sigma \cdot \sigma', i = 1, \dots, \nu - k).$$

Note that  $\phi_i(p) = \xi_{\sigma, \sigma'}(p, e_i)$  ( $p \in \text{any } \sigma'^{-1}$ ,  $i = 1, \dots, \nu - r + 2$ ) defines orthogonal projections of  $K'^{-1}$  into  $\mathfrak{S}$ .

4. *Characteristic Classes*.—Choose  $\phi_1, \dots, \phi_{\nu-r+2}$  as above over  $K'^{-1}$ ; then for each  $\sigma'$ , studying these on  $\partial\sigma'$  gives  $W' \cdot \sigma'$ , which is an integer mod 2 if  $r = 1$  or  $r \leq \nu$  is even, and an integer otherwise.  $W'$  is a cocycle whose class  $\mathbf{W}'$  is an invariant of  $\mathfrak{B}$ ; the  $\mathbf{W}'$  characterize  $\mathfrak{B}$  if  $\nu \leq 1$  or  $\dim(K) \leq 3$  (see TP). We may use a general type of subdivision of the polyhedron  $K$  in defining the  $\mathbf{W}'$ .

If  $\mathfrak{B}$  is not orientable (TP, §4), and  $K'$  is obtained from  $K$  by replacing  $[\sigma': \sigma'^{-1}]$  by  $-[\sigma': \sigma'^{-1}]$  when  $\xi_{\sigma, \sigma'}$  and  $\xi_{\sigma, \sigma'+1}$  give opposite orientations to the  $S(p)$  ( $p \in \sigma'$ ) (see TP, p. 793, footnote), then  $K'$  is locally isomorphic with  $K$ , and  $\mathbf{W}' = \mathbf{W}^1$ . We call  $K'$  the *complex associated with*  $\mathfrak{B}$ . The characteristic classes are taken in  $K'$ ; the theorems above hold still.

If  $f$  maps  $K_1$  into  $K_2$ , and  $\mathfrak{B}_2(K_2)$  is defined, then a bundle  $\mathfrak{B}_1(K_1)$  is defined (TP, §8), and  $\mathbf{W}_1' = f' \mathbf{W}_2'$  ( $f' = \text{dual of } f$ ).

If  $\nu = 2$ ,  $\dim(K) = 4$ , and  $\mathbf{W}^1 = 0$ ,  $\mathbf{W}^2 = 0$ , then an invariant characterizing  $\mathfrak{B}$  is obtained as follows. A triple  $\phi = (\phi_1, \phi_2, \phi_3)$  of orthogonal projections of  $K^3$  into  $\mathfrak{S}(K^3)$  exists. Let  $\Phi(p)$  ( $p \in K^3$ ) map  $S_0$  into  $S(p)$  so that  $\Phi(p, e_i) = e_i$  ( $i = 1, 2, 3$ ). For each  $\sigma^4$ , set

$$\Psi_{\sigma^4}(p) = \xi_{\sigma^4}^{-1}(p) \Phi(p) \quad (p \in \partial\sigma^4).$$

This maps  $\partial\sigma^4$  into the orthogonal group  $G^3$ ; as  $G^3$  is homeomorphic with projective 3-space  $P^3$ , this defines an integer  $D^4 \cdot \sigma^4$ , the degree of  $\Psi_{\sigma^4}$ .  $D^4$  is a cocycle. If we identify two cocycles if they are cohomologous, or differ by a cocycle of the form  $X^1 \smile X^1 \smile X^1 \smile X^1$  ( $X^1$  a 1-cocycle), the class determined is the invariant.

The classes  $W^{2r+1}$  are determined from the others as follows (see I, §11):

$$W^{2r+1} = \frac{1}{2} \partial \omega W^{2r} \quad (\text{if } \nu \geq 2r).$$

5. *On Mappings into  $G^{\nu+1}$* .—In the theorem just stated, and in the duality theorem, we need the following (and other more complicated) theorems. (a) Let  $f$  map  $\sigma'$  into  $G^{\nu+1}$  so that if  $\phi(p) = f(p, e_1)$ , then  $\phi(p) = e_1$  in  $\partial\sigma'$ ; let  $\phi$  be of degree  $\alpha$ . Let  $\psi$  map  $\sigma'$  into  $S_0''$  with the degree  $\beta$ , and let  $\psi(p) = e_1$  ( $p \in \partial\sigma'$ ). Then  $\theta(p) = f(p, \psi(p))$  is of degree  $\alpha + \beta$ . (b) Take  $f$  as before; then  $\phi'(p) = f^{-1}(p, e_1)$  (the point of  $S_0$  mapped into  $e_1$  by  $f(p)$ ) is of degree  $-\alpha$ . (Use (a).) (c) Let  $f$  map  $\sigma'$  into  $G^{\nu+1}$ , let  $\phi$  map  $\partial\sigma'$  into  $S_0'^{-1}$  with the degree  $\alpha$ , and suppose  $f(p, \phi(p)) = e_1$  ( $p \in$

$\partial\sigma'$ ). Then  $\psi(p) = f(p, e^{r+1})$  maps  $\partial\sigma'$  into the  $S_1^{r-1}$  orthogonal to  $e_1$  with a degree  $\equiv \alpha \pmod{2}$ .

6. *The Duality Theorem.*—Given bundles  $\mathfrak{B}_1^\lambda(K)$  and  $\mathfrak{B}_2^\mu(K)$ , there is a uniquely determined bundle  $\mathfrak{B}_3^r(K)$ , their *product* ( $r = \lambda + \mu + 1$ ; see TP, p. 796); thus if  $M^m \subset M^n$ , the tangent times the normal bundle gives the part of the tangent bundle of  $M^n$  over  $M^m$ . The formula for the characteristic classes of  $\mathfrak{B}_3^r$  is

$$W_3^r = \sum_i W_1^i \smile W_2^{r-i}, \text{ reducing mod 2 if necessary.}$$

(See §4 and I, §12; we use  $W^0 = \text{sum of vertices}$ .) The proof is very difficult if  $r \geq 4$ . We use the special  $\xi_{1,r}$  of §3 in  $\mathfrak{B}_1$ , and  $\xi_{2,r}$  in  $\mathfrak{B}_2$ , with  $a$  replaced by  $a' < a$ , so the  $P_1$  and  $P_2$  will be in "general position" (see I, §5). The projections into  $\mathfrak{B}_3(K^{r-1})$  are defined successively over  $Q_1^0, Q_1^1, \dots$ . For each  $\sigma'$ , they are now deformed in  $\partial\sigma'$  into a simpler position, except in each  $A^i = P_1^i \cdot P_2^{r-1-i} \cdot \sigma'^{-1}$  ( $\sigma'^{-1} = \text{face of } \sigma' \text{ opposite first vertex of } \sigma'$ ). The terms shown come from the  $A^i$ , two coming from  $A^{r-1}$ . The results of §5 and the products of I, §6, are needed.

REMARK. We do not know whether or not the individual terms  $W_1^i \smile W_2^{r-i}$  have topological significance.

Reducing everything mod 2, write, for any  $\mathfrak{B}$ , the formal power series

$$W = \sum_i W^i t^i, \quad \bar{W} = 1/W = \sum_i \bar{W}^i t^i;$$

then

$$\bar{W}^0 = I|_2, \quad \bar{W}^1 = W^1, \quad \bar{W}^2 = W^2 + W^1 \smile W^1,$$

etc. The duality theorem gives then, as  $W_N = W/W_T$ , etc.,

$$W_{N'} = \sum W^i \smile \bar{W}_T^{r-i}, \quad \bar{W}' = \sum \bar{W}_T^i \smile \bar{W}_{N'}^{r-i}, \text{ etc. } \pmod{2}.$$

7. *Tangent Bundles.*—Let  $K$  be a simplicial subdivision of the manifold  $M^n$ , with ordered vertices. Each  $p$  in  $K$  may be written uniquely as  $p = \sum \eta_{\lambda_i}(p)x_{\lambda_i}$ , if  $p \in x_{\lambda_0} \dots x_{\lambda_r}$ . Define

$$v_k(p) = \sum_{\lambda_0 < \dots < \lambda_k} \eta_{\lambda_0}(p) \dots \eta_{\lambda_k}(p)(x_{\lambda_k} - x_{\lambda_{k-1}}) \quad (k = 1, 2, \dots).$$

These are continuous in  $K$ , and the first  $r$  are independent except in  $K^{r-1}$  (any  $r$ ). If  $K^*$  is the usual complex dual to  $K$ , these may be used to define  $W^r$ , a cocycle in  $K^*$ . Its dual is a *characteristic cycle*  $C^{n-r}$  in  $K'$ , the complex associated with  $\mathfrak{B}$  (which was studied in I, §13). Note that  $C^{n-(r+1)} = \frac{1}{2}\partial\omega C^{n-r}$ . The value of  $C^s \cdot \sigma^s$  ( $s = n - r$ ,  $\sigma^s = x_{\lambda_0} \dots x_{\lambda_s}$ ) is as fol-

lows. Let  $K_1$  be the subcomplex of the closed star of  $\sigma^s$  containing all vertices  $x_i$  with

$$\lambda_s > i > \lambda_{s-1} \text{ or } \lambda_{s-2} > i > \lambda_{s-3} \text{ or } \dots$$

(This includes all vertices below  $\lambda_0$  if  $s$  is even.) Then  $C^s \cdot \sigma^s = 1 - \chi(K_1)$  ( $\chi$  = Euler-Poincaré characteristic), or this mod 2.

From this we prove: If  $K$  is the first derived of a simplicial subdivision of  $M$ , then  $C^s$  is the sum of all  $s$ -simplexes of  $K$  (properly oriented if integer coefficients are used).<sup>2</sup>

In the proofs of the following theorems, we study the classes over submanifolds of the given manifold, and use the duality theorem and results from §8. For  $M^m \subset M^n$ , let  $\mathbf{W}^r$  mean the part of  $\mathbf{W}_T^r(M^n)$  in  $M^m$ .

If a closed  $M^m$  can be imbedded in  $E^n$  (with or without singularities), then

$$0 = \mathbf{W}_N^{n-m}|_2 = \sum \mathbf{W}^i \smile \overline{\mathbf{W}}_T^{n-m-i} = \overline{\mathbf{W}}_T^{n-m}.$$

Hence  $\overline{\mathbf{W}}_T^m = 0$  always. This gives, if  $(X)^2 = X \smile X$ , etc.,

$$\text{closed } M^2: \mathbf{W}^2|_2 = (\mathbf{W}^1)^2; \text{ closed } M^3: (\mathbf{W}^1)^3 = 0;$$

$$\text{closed } M^4: \mathbf{W}^4|_2 + (\mathbf{W}^2)^2 + \mathbf{W}^2 \smile (\mathbf{W}^1)^2 + (\mathbf{W}^1)^4 = 0; \text{ etc.}$$

In any  $M^2$ , for any 1- $I_2$ -cocycle  $X^1$ ,  $X^1 \smile X^1 \smile X^1 \smile W^1$ . In any  $M^3$ ,  $\mathbf{W}^2 = \mathbf{W}^1 \smile \mathbf{W}^1$ ; hence (Stiefel) for orientable  $M^3$  (closed or not), the tangent bundle is simple. In any orientable  $M^4$ ,  $\mathbf{W}^3 = 0$ . (The proof uses facts from §10.) For any 2- $I_2$ -cocycle  $X^2$  in any  $M^4$ ,  $X^2 \smile X^2 \smile X^2 \smile \overline{\mathbf{W}}^2$ . For any orientable  $M^4$  in an orientable  $M^r$ ,  $\overline{\mathbf{W}}_T^4 = \mathbf{W}_N^4$ .

8. *Normal Bundles*.—For any  $M^m$  in any  $M^n$ ,  $\mathbf{W}_N^{n-m}$  is the intersection of  $M^m$  with itself in  $M^n$ , which is a cohomology class of the complex associated with the normal bundle; if  $M^m$  is closed and  $M^n = E^n$ , then  $\mathbf{W}_N^{n-m}|_2 = 0$ , and if also  $M^m$  is orientable, then  $\mathbf{W}_N^{n-m} = 0$ . Compare PC, §20, and TP, p. 795.

If  $M^m$  is mapped regularly into  $M^n$ , but with singularities, we may deform  $M^m$  slightly into  $M'^m$ , and consider the intersections of the  $\sigma'$  with a neighborhood of  $\sigma'$  in  $M$ ; then  $\mathbf{W}^{n-m}$  is the *local intersection* thus defined. For a closed orientable  $M^m \subset E^n$ , the *intersection* vanishes, so that the *distant intersection* equals the local. This holds mod 2 in the non-orientable case. If  $n = 2m$ , and the singularities are isolated points, the distant intersection is of course 0 (mod 2); if  $m$  is odd, it vanishes, because  $\{\sigma^m, \sigma'^m\} = -\{\sigma'^m, \sigma^m\}$ .

Take an orientable  $M^m \subset E^n$ . Then the normal bundle is simple if  $m = 1$  or 2, or  $n = m + 1$  or  $m + 2$ , or  $m = 3$  and  $M$  is closed, or  $M$  is a cell. This holds if  $M^m$  is merely mapped regularly, provided that if  $n = m + 2$ , then  $m$  is odd, and we omit  $m = 2$  if  $M$  is closed.

9. *Examples*.—Consider a cylinder, the product  $T^2 = T^1 \times T^1$  of a segment and a disc. Let  $P^2$  be one end, let  $S_1^1$  be a segment crossing  $P^2$ ,

let  $Q^2$  be a rectangle cutting through  $T^3$  and ending on  $S_2^1$ , and let  $S_1^1$  be the center of  $Q^2$ , ending at  $p_0$ , the center of  $S_2$ ; rather, let these be the sets after the identifications below. (1) Join the ends of  $T^3$ , and shrink  $\partial T^2$  to a point; this forms  $M_1^3 = S^1 \times S^2$ , with a simple tangent bundle. (2) Join the ends, and identify opposite points of  $\partial T^2$ ; then characteristic cycles are (mod 2)  $C^2 = Q^2$ ,  $C^1 = S_1^1$ ;  $\therefore \mathbf{W}^2 \neq 0$ . (3) Join the ends, reflecting one so that  $P^2$  is joined to itself with orientation reversed, and shrink  $\partial T^2$  to a point. We obtain  $M_3^3$ , with  $\mathbf{W}^2 = 0$ . (4) Join the ends as in (3), and identify opposite points of  $\partial T^2$ , forming  $M_4^3$ . Now  $P^2$  is a projective plane, and  $Q^2$  is a Klein bottle. Intersections are (mod 2)

$$\{P^2, P^2\} \sim 0, \{Q^2, P^2\} \sim S_2^1, \{Q^2, Q^2\} \sim S_1^1 + S_2^1;$$

$$\{P^2, S_2^1\} \sim 0, \{P^2, S_1^1\} \sim p_0, \{Q^2, S_2^1\} \sim p_0, \{Q^2, S_1^1\} \sim 0;$$

characteristic classes are

$$C^2 \sim (P^2 + Q^2)_2, C^1 \sim (S_1^1 + S_2^1)_2.$$

Define  $\mathfrak{B}_1^1(M_4^3)$ , with  $C_1^2 \sim P^2$ ,  $C_1^1 \sim 0$ , and  $\mathfrak{B}_2^1(M_4^3)$ , with  $C_2^2 \sim Q^2$ ,  $C_2^1 \sim 0$ ; let the total spaces be  $M_1^4, M_2^4$ . We may pretend  $M_4^3$  is in either (because  $C_i^1 = 0$ ). Then

$$C_1^3 \sim \mathfrak{S}(Q^2), C_1^2 \sim \mathfrak{S}(S_1^1), C_1^1 \sim \mathfrak{S}(p_0); \therefore \overline{C}_1^1 \sim 0;$$

$$C_2^3 \sim \mathfrak{S}(P^2), C_2^2 \sim \mathfrak{S}(S_2^1), C_2^1 \sim \mathfrak{S}(p_0); \therefore \overline{C}_2^1 \sim \mathfrak{S}(p_0);$$

hence in  $M_2^4$ ,  $\mathbf{W}^3 \neq 0$ ,  $\overline{\mathbf{W}}^3 \neq 0$ . Hence (see §7)  $M_2^4$  cannot be imbedded in  $E^7$ .

Define  $\mathfrak{B}^2(M_3^3)$ , with  $C^2 \sim P^2$ , and  $C^1 \sim S_1^1$ . Then we may consider  $M_3^3 \subset M^5 = \mathfrak{S}(M_3^3)$ , and prove (a)  $M^5$  is closed and orientable, (b)  $C^2(M^5) \sim \mathfrak{S}(p)$ ; hence  $\overline{\mathbf{W}}^3 = \mathbf{W}^3 \neq 0$ , and  $M^5$  cannot be imbedded in  $E^8$ .

We may define  $M^5 = \mathfrak{S}(S_0^4)$ , with  $\overline{\mathbf{W}}^4 = \mathbf{W}^4 \neq 0$ ; hence  $M^5$  cannot be imbedded in  $E^{12}$ .

The complex projective plane  $P^{*4}$  cannot be imbedded in  $E^6$ , as  $\mathbf{W}^2 \neq 0$ . ( $\mathbf{W}^2 \smile \mathbf{W}^2 = \mathbf{W}^4|_2$ ;  $\mathbf{W}^4 \cdot P^{*4} = \chi(P^{*4}) = 3$ .) But it can be in  $E^7$ .

For any closed orientable  $M^4$ , and any cocycles  $X^3, X^4$  (mod 2) in  $M^4$ , we may imbed  $M^4$  in an  $M^8 \subset E^{17}$ , so that the part over  $M^4$  of the characteristic classes of the normal bundle of  $M^8 \subset E^{17}$  are  $X^3$  and  $X^4$ . Hence we may make  $\mathbf{W}_N^3 \neq 0$  and  $\mathbf{W}_N^4 \neq 0$  also.

If we put a Klein bottle  $Q^2$  in  $E^3$ , then the distant and local intersections are equal (mod 2); these are  $\mathbf{W}_N^1 = \mathbf{W}_T^1$ ;  $C_T^1$  = a closed curve in  $Q^2$ . Hence the distant intersection, as a cycle, is a certain curve in  $Q^2$ , as is clear in the usual immersion of  $Q^2$  in  $E^3$ . For  $P^2$  in  $E^3$ , we get the "projective line" similarly.

A direct study shows: If  $M^2 \subset E^4$  is closed (with or without singularities), and  $C^2$  is the fundamental cycle of the associated complex (integer



coefficients), then  $W^2 \cdot C^2 = 2[\chi(M^2) + 2k]$  for some  $k$ ; any  $k$  may be obtained. Hence if  $\chi \not\equiv 0 \pmod{2}$ , then a field of normal vectors never exists. Also one imbedding cannot be deformed into another with a different  $W^2 \cdot C^2$ .

10. *Homology Groups of Total Spaces, Etc.*—Let  $K$  be connected. Given  $\mathfrak{B}(K)$ , the homology groups satisfy  $\mathbf{H}^r(\mathfrak{S}) \approx \mathbf{H}^r(K)$ ,  $r < \nu$ .  $kS(p) \sim 0$  if and only if for some  $A^{\nu+1}$ ,  $A^{\nu+1} \cdot W^{\nu+1} = k$ . (We may use  $k_\lambda = k \pmod{\lambda}$ , and  $A^{\nu+1}|_\lambda$ .) For  $\mathfrak{B}$  oriented, a  $(\nu+1)$ - $I_\mu$ -cycle  $A$  in  $K$  is the projection of such a cycle in  $\mathfrak{S}$  if and only if  $A \cdot W^{\nu+1} = 0_\lambda$ . Now  $\mathbf{H}^r(\mathfrak{S})$  may be described in terms of  $\mathbf{W}^{\nu+1}$  and properties of  $kS(p)$ . If  $\mu$  is the smallest integer such that  $\mu S(p) \sim 0$ , then  $\mathbf{H}^r(\mathfrak{S}) \approx I_\mu \oplus \mathbf{H}^r(K)$  if and only if for each  $\lambda$  and each  $(\nu+1)$ - $I_\lambda$ -cycle  $A$ ,  $A \cdot W^{\nu+1} \equiv 0 \pmod{(\lambda, \mu)}$ . A mapping  $f$  of a complex  $K'$  of dimension  $\leq \nu+1$  into  $K$  is the projection of a mapping into  $\mathfrak{S}$  if and only if  $f'W^{\nu+1} = 0$ .

<sup>1</sup> We refer the reader to papers in these PROCEEDINGS, 21, 464–468 (1935), and in *Bull. Am. Math. Soc.*, 43, 785–805. We denote the latter by TP, and the preceding note, by I. Sphere-bundles were formerly called “sphere-spaces.”

<sup>2</sup> This was a conjecture of Stiefel, *Comm. Math. Helv.*, 8, 40 (1936).



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## *THE EXPERIMENTAL PRODUCTION OF MELANIN PIGMENT ON THE LOWER SURFACE OF SUMMER FLOUNDERS (PARALICHTHYS DENTATUS)<sup>1, 2</sup>*

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Differences in the degree of pigmentation on the upper and lower surfaces of animals have for centuries attracted man's interest. Such differential pigmentation, although perhaps most markedly exemplified in the lower vertebrates (fishes and amphibians), is seen also to a lesser extent in all the other vertebrate classes and even in many invertebrates.

Flounders provide an excellent example of differential pigmentation as they have entirely unpigmented lower sides but densely pigmented upper surfaces. They are doubly interesting because as larvae they display bilateral pigmentation which disappears on one side coincident with the migration of one eye and the secondary or adult orientation of the fish in a plane at right angles to the original (Agassiz, 1878). Naturally enough, Cunningham (1891, 1893, 1895) associated light with the presence of pigmentation and, on this basis, illuminated larval flatfishes ventrally to see if the bilaterally pigmented pattern would be retained even after the metamorphosis of the fish and the secondary orientation of the body with one flat surface against the substrate. Although he failed to retain the original bilateral pattern, it was found in a fair percentage of cases that after several months of ventral illumination some pigment did develop on the normally unpigmented lower side. However, Agassiz (1878) reported no development of ventral<sup>3</sup> pigment in flounder larvae which were exposed to light ventrally for the express purpose of arresting the migration of the eye in metamorphosis.

<sup>1</sup> This work was aided in part by a Bache Fund grant administered by Professor G. H. Parker.

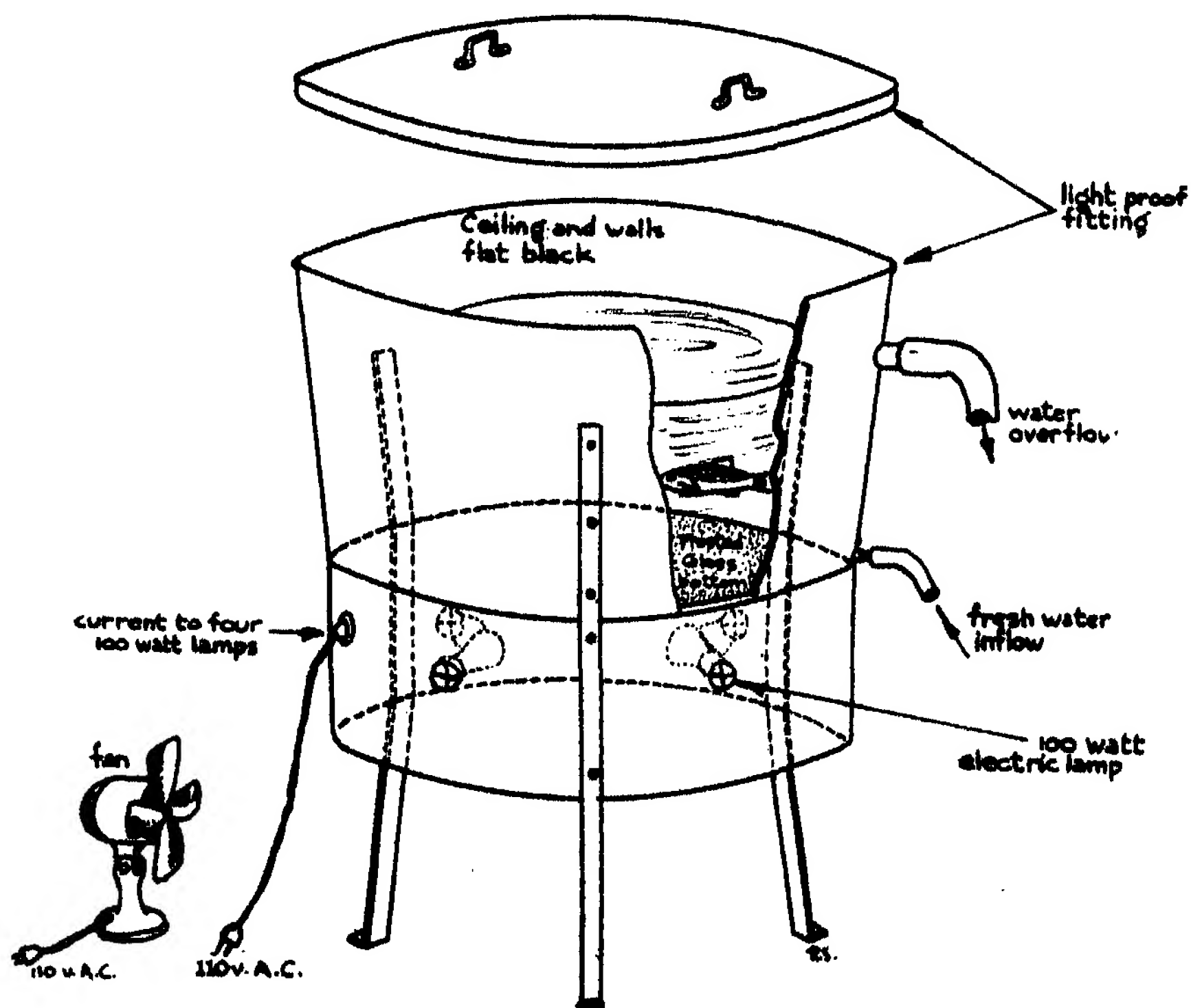
<sup>2</sup> Contribution No. 247 of the Woods Hole Oceanographic Institution whose research facilities were generously provided for this investigation.

<sup>3</sup> The term "ventral" will be used in this paper to indicate the lower surface of a naturally oriented fish.

This paper presents descriptive data on preliminary experiments designed to show the relationship between directed continuous illumination, vision and the production of melanin pigment.

*Materials and Methods.*—The experimental flatfishes, summer flounders (*Paralichthys dentatus*) 11 to 17 inches long, were taken by otter trawl from Woods Hole waters. Live cars of neutral shade were anchored in the harbor for storing stock animals but most of the fishes used were freshly

**FIG. A, EXPERIMENTAL SETUP**  
**PROVIDING VENTRAL ILLUMINATION**



netted. Ventral illumination was provided by an apparatus similar to that shown in Fig. A. The temperature of the running sea water averaged 19°C. throughout these experiments. Some of the fishes were blinded by complete optic enucleation and in others both optic nerves were cut. Large experimental tanks painted black or white inside and illuminated continuously from above were used for extreme (black or white) back-

ground adaptation. Numerous controls were kept for each experimental situation.

*Experiments and Observations:*

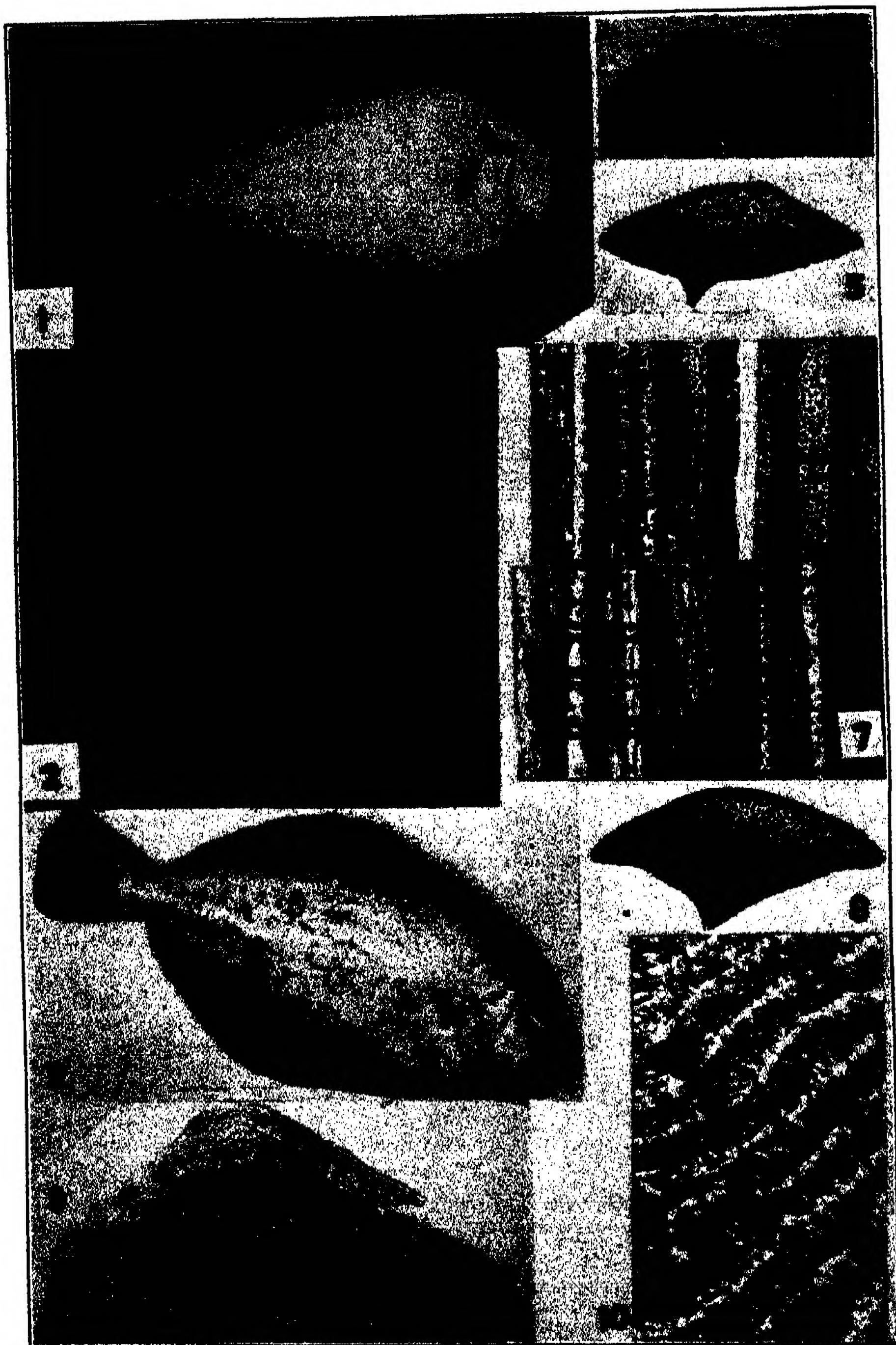
1. *Unoperated Flounders with Lower Surface Illuminated.*—Eight freshly caught summer flounders were, on different occasions, placed in the apparatus providing a diffuse direct illumination of the lower side for periods ranging from seven to 51 days. Pigment developed to some extent on the normally pale surface (Fig. 1) of all of these fishes. In one flounder the first pigment was apparent after seven days but only after 15 to 25 days of ventral illumination was a melanin development obvious in most of the animals. After these initial stages the macroscopic increase in melanin was more rapid. A growth comparable to that in figures 2 and 3 was attained in about seven weeks. The general body shade (upper surface) of animals kept in the apparatus (black side walls and ceiling) was definitely darker than intermediate but yet not fully black-adapted. They were more nearly dark brown than black. Paler contrasty spots were frequently observed.

2. *Blinded Black Flounders Illuminated on Lower Surface.*—Five black-adapted flounders were blinded and placed in the apparatus providing ventral illumination. All of these fishes developed ventral pigment which first became apparent in 12 to 14 days and became pronounced in 45 days.

3. *Unoperated Flounders on Black Background Illuminated from Above.*—Another set of 12 unoperated fishes was placed in an experimental tank illuminated from above. The floor and side walls were flat black and the light source was of the same intensity as in the previous experiments. The animals became black-adapted in a few hours (Osborn '39a), finally reaching a fully black shade which persisted throughout the experiment. Typical white excitement spots could be elicited at any time upon application of the appropriate stimulus (Osborn '39a). The fishes remained in this situation for periods ranging from 15 to 70 days but none developed ventral pigment.

4. *Blinded Black Flounders on Black Background Illuminated from Above.*—Experiment 3 was repeated with ten flounders but this time the animals were totally blinded as soon as they were fully black-adapted. Such fishes were maintained under these conditions as long as 56 days but ventral pigment did not develop.

5. *Blinded Black Flounders on White Background Illuminated from Above.*—Summer flounders, 14 in all, were totally blinded following black-adaptation and were then transferred to white experimental tanks continuously illuminated from above. Flounders thus prepared remain maximally dark even on the white background (Osborn '39a). The light source was of the same intensity as employed above but the resulting illumination much brighter due to the high incidence of reflection from the





white floor and walls of the tank. Under these conditions, some evidence of developing ventral pigment appeared as soon as 15 days but the process was very slow and only a small amount developed in 60 to 70 days. Longer periods of treatment would undoubtedly result in increased pigmentation. However, the unmistakable amounts of ventral pigment developing in every fish suggest that vision is not essential to this process. On the contrary, normal unoperated control fishes placed under similar conditions at the same time failed to develop any ventral pigment and, of course, became typically white-adapted on the upper surface (Osborn '39a). Furthermore, it has been shown in fundulus and catfish that prolonged white-adaptation actually results in melanophore degeneration and an absolute decrease in the melanin of the skin (Odiorne '37).

6. *Blinded White Flounders on White Background Illuminated from Above.*—Nine fishes were completely white-adapted (seven days) and then totally blinded and replaced in illuminated white tanks under conditions identical with those of experiment No. 5. Summer flounders so prepared do not darken quickly and maximally as do many fishes but rather darken very slowly to an intermediate shade (Osborn '39a and b). Of nine such animals, six failed to develop appreciable amounts of ventral pigment in 40

## PLATE I

## EXPLANATION OF FIGURES

Figure 1. Lower unpigmented surface of a normal summer flounder which was removed from nature and photographed immediately. The upper surface was in the dark phase.  $\frac{1}{8}$  natural size.

Figure 2. Lower surface of a summer flounder which had been illuminated ventrally for seven weeks in the apparatus in figure A. Note that considerable pigment has developed.  $\frac{1}{8}$  natural size.

Figure 3. Same as figure 2 but on white background to show the developed pigment in better contrast.

Figure 4. Photomicrograph of the exposed surface of a typical scale plucked from the lower surface of a normal untreated fish. Note that no trace of pigmentation is apparent.  $\times 25$ .

Figure 5. Scale plucked from upper normally pigmented surface of a summer flounder. The scale surface is quite fully covered by melanophores.  $\times 25$ .

Figure 6. Portion of the pectoral fin taken from the pigmented side of a control fish. It is highly pigmented.  $\times 25$ .

Figure 7. Portion of the pectoral fin removed from the lower normally unpigmented surface of a fish which received ventral illumination for 6 weeks. This treatment has produced marked pigmentation.  $\times 25$ .

Figure 8. A scale plucked from the lower surface of a fish illuminated ventrally 7 weeks. Note that it is pigmented as fully as is the dorsal scale in figure 5.  $\times 25$ .

Figure 9. A scale from the lower surface of a fish illuminated ventrally for 3 weeks. Macroscopically only initial traces of pigmentation could be detected. Note that the melanophores are sparsely distributed.  $\times 25$ .

Figure 10. An area of the scale in figure 9 showing the details of the newly developed melanophores.  $\times 140$ .

to 50 days. The other three fishes exhibited early stages of melanophore development on a few scales. This experiment must be repeated on more animals and be allowed to run three or four months.

7. *Blinded Flounders on Illuminated Backgrounds*.—Seven summer flounders were totally blinded as soon as they were taken from nature. They were an intermediate greenish brown at the time. After blinding, they darkened slightly to a very homogeneous deep chocolate-brown and were placed in an illuminated *white* experimental tank as in the previous experiments. These fishes developed appreciable ventral pigment in 38 days and definitely more in 50 to 60 days. Two other fishes received similar treatment in all details but were placed in an illuminated *black* experimental tank. Pigment did not develop on the lower surface of either of these fishes in 52 days.

*Discussion*.—Perhaps Agassiz failed to get pigmentation because his light source (daylight) was not directed onto the lower surface of the fishes. They were simply placed in glass-bottomed dishes near the window. Since daylight varies in intensity with time of day and the weather, it would necessarily take two or three times as long for positive results as would continuous artificial illumination of constant high intensity. Agassiz, who was primarily interested in the migration of the eye in metamorphosis, probably did not continue his experiments long enough to grow pigment.

Cunningham designed his experiments for pigment studies, continued them for periods of from several months to over a year and reflected daylight by mirrors directly onto the lower surfaces of his fishes.

The confirmatory results presented here were obtained in relatively shorter periods probably because the light was of higher intensity and directed continuously onto the animals. These flounders were also larger and older than those used by previous investigators.

The artificially produced pigment is melanin in melanophores (Figs. 7, 8, 9 and 10) which appear to be normal morphologically and physiologically. When ventral scales bearing these melanophores are placed in adrenalin a typical concentration of the pigment granules occurs. Furthermore, when the scale is plucked and the nervous connections severed, the melanophores typically exhibit maximal expansion.

The source of these new melanophores is an unsettled question. Two possibilities are indicated: either they differentiate from some other cell already at the site or they migrate in from other areas. In the latter case they might migrate as typical melanophores or as cells capable of becoming melanophores. Experiments designed to provide more information on these points are now in progress. Thus far, no evidence for the migration of melanophores to the unpigmented area has been obtained. Cunningham, failing to find evidence to the contrary, believed that the pigment cells developed *in situ*.

*Summary.*—An apparatus providing continuous artificial illumination of constant intensity directed to the lower surface of flounders is pictured. Pigmentation was developed on the lower normally unpigmented surface in a high percentage of summer flounders in the following experimental situations: (1) Unoperated fishes in black tanks illuminated from below. (2) Blinded dark fishes in black tanks illuminated from below or in white tanks illuminated from above.

The observation that flounders blinded in the dark phase developed ventral pigment as readily as unoperated ones indicates that the eyes are not essential to this reaction.

Light is a necessary factor in the production of ventral pigment.

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## A RESPIRATORY PIGMENT FROM THE EGGS OF A MARINE WORM

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Of the wide diversity of pigments occurring in nature, a certain number are considered to function as respiratory carriers by virtue of their ability to be reversibly oxidized and reduced (see review of Barron<sup>2</sup>). I wish to report here the presence of such a pigment in the eggs of the marine worm *Urechis caupo*, together with evidence for its probable participation in cellular respiration.

The eggs of *Urechis caupo* are typically pink in color. In small, or relatively unripe, females, however, it is frequently found that the eggs are not pink, but yellow. Although the eggs of any one individual are all of the same color, a comparison of the eggs from different individuals shows

an intergradation of color from light yellow to an intense pink. It was found that if yellow eggs are placed in a Thunberg tube, and the tube then evacuated, they gradually become pink. If the vacuum is then broken and the eggs aerated, they regain their yellow color. The development of a pink color is apparently the result of the reversible reduction, *in vivo*, of a pigment present in yellow and pink eggs alike. The pigment in pink eggs can be oxidized by adding a small amount of  $H_2O_2$  to the suspension (plus a trace of HCN to inhibit the powerful catalase contained in the cells). They thereupon become yellow. If the eggs are then washed and placed in an evacuated Thunberg tube they become pink again.

To obtain the pigment from the cells, pink eggs are extracted with acetone for 3–6 hours in a Soxhlet apparatus. This removes large quantities of two yellow pigments—one water-soluble and the other fat-soluble. The pink pigment remains behind. The nature of the two acetone-extractable pigments is as yet uncertain. Neither of them, however, shows reduction to a pink form. The pink pigment is then extracted by shaking with 5% HCl-methanol at 40°C. The pigment thus extracted consists of a mixture of the reduced form and its yellow oxidation product. If the extract is placed in the icebox overnight, the reduced form largely precipitates out in dark red, amorphous particles. The supernatant, containing the oxidized pigment and a small amount of the reduced pigment, is concentrated by distillation *in vacuo* and is finally dried on a water bath.

The oxidized form of the pigment is readily soluble in water. It is reduced by hydrosulfite, or by hydrogen in the presence of a platinum catalyst, to a pink (in concentrated solution, red) pigment. Upon shaking with air it reoxidizes to the yellow form. Autoxidation in air occurs rapidly at neutral and alkaline pH's. The reduced form is only sparingly soluble in acid solution (<pH 5.5). It is readily soluble at neutral and alkaline pH's, but immediately autoxidizes if oxygen is present. The pigment is rapidly destroyed by strong alkali. Autoxidation of the pigment can be accelerated *in vivo* by raising the intracellular pH by means of the penetrating base ammonia. Pink eggs so treated become yellow. Upon washing away the ammonia, the pink color returns.

The oxidation-reduction potential of the pigment has been determined polarographically<sup>3</sup> through the coöperation of Professor J. Percy Baumberger, using the purest preparation so far obtained. At pH 7.39  $E'_0 = +0.163$  volt (250°C.).  $E'_0$  decreases 0.059 volt per unit increase in pH in the pH range 5–10. The change in  $E_h$  with change in degree of oxidation corresponds to a one-electron process. These results will be presented in detail in a future communication.

The facts that the pigment occurs naturally in both oxidized and reduced states, and that it is reducible by the cells and autoxidizes in the physiological range of pH, indicate that it is probably involved in the

cellular respiration. It is suggested that the pigment be called *urechrome* (not to be confused with the urinary pigment, urochrome). The chemical nature, absorption spectrum and physiological function of the substance are being studied.

<sup>1</sup> National Research Council Fellow in Zoölogy.

<sup>2</sup> Barron, E. S. G., *Physiol. Rev.*, **19**, 184-239 (1939).

<sup>3</sup> Müller, O. H., and Baumberger, J. P., *Trans. Electrochem. Soc.*, **71**, 169-194 (1937).

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### FURTHER STUDIES ON THE PARTHENOGENETIC ACTIVATION OF RABBIT EGGS\*

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In the course of certain studies on the artificial activation of rabbit tubal ova *in vitro* in which the development in culture of ova given certain stimulating treatments was contrasted with the development of untreated ova, we noted that in certain of the control cultures some ova gave clear evidence of activation. Our experiments were being conducted in a basement room at temperatures ranging between 17°C. and 21°C. Furthermore a period of one to two hours often elapsed between the sacrifice of the donor of the ova and their final incubation at 37.5°C. It seemed possible, therefore, that the cooling of rabbit ova might lead to activation. Some 80 unfertilized ova were cooled either by keeping them at room temperature for 2 to 3½ hours (34 eggs) or by placing them in a refrigerator (at 6°C.) for 15 to 85 minutes (46 eggs). These ova, like all the others in our experimental series (see table 1), were cultured for 20 to 24 hours in rabbit serum at 37.5° (see Shapiro<sup>2</sup>), then fixed in Bouin's fluid (Pincus<sup>1</sup>), sectioned and stained with Ehrlich's hematoxylin, and examined for cytological evidences of activation.

In table 1 we present a summary of our data on the effects of cooling and also on the effects of exposing ova to: (1) balanced salt solutions made hypotonic by dilution with glass distilled water (usually 1 part salt solution to 1 part distilled water), (2) rabbit serum diluted to one-half by distilled water, (3) hypertonic balanced salt solutions (1.6 to 1.8% salt) and (4) hypertonic and hypotonic solutions alternately. Ova are considered activated when they exhibit clear pronuclei, or cleavage chromosomes, or cleavage. Ova classified as not activated either showed marginal meiotic



chromosomes (as at ovulation), or subnuclei (due to the scattering of meiotic chromosomes and nucleus reformation about single chromosomes or groups), or cytoplasmic fragmentation without cleavage. In some instances ova showed true cleavage had occurred followed or accompanied by cytoplasmic fragmentation—such eggs were considered activated.

The cooled ova appear to have been activated (and proceeded to cleavage) in a larger proportion than the other ova of these series. Actually our data indicate that cooling at 6°C. for 10 to 30 minutes was most effective: of 33 such ova 13 were activated and 10 cleaved, 7 of these cleavages without any cytoplasmic fragmentation. Of 13 ova placed at 6°C. for 85 minutes, 6 cleaved but 5 of these 6 showed cytoplasmic fragmentation.

We decided to attempt to cool ova *in situ* in the fallopian tubes. At 14 to 19 hours after the injection of an ovulating pituitary extract (Pincus<sup>1, 2</sup>)

TABLE 1

THE EFFECTS OF VARIOUS TREATMENTS ON UNFERTILIZED RABBIT OVA CULTURED *in vitro* FOR 20 TO 24 HOURS

TREATMENT	NUMBER OF EGGS	NUMBER ACTIVATED	NUMBER CLEAVED	% ACTIVATED	% CLEAVED
Controls, no treatment	143	20	11	14.0	7.9
Cooling	80	42	19	52.5	23.8
Hypotonic balanced salt solutions	92	37	16	29.3	17.4
Hypotonic serum	354	134	32	37.9	9.0
Hypertonic balanced salt solutions	24	7	2	29.2	8.3
Alternating hypertonic and hypotonic solutions	29	9	2	31.0	7.0

the ovulated ova are massed below the first loop of the fallopian tube in a narrow portion and ordinarily so distend the tube as to be visible as a translucent bulge. We designed a hollow brass jacket which would enclose 3 centimeters of the tube at this point. Laparotomy was performed under combined ether and nembutal anesthesia, the sterilized cooling jacket placed about the right fallopian tube, and ice water circulated through the cooling jacket for appropriate periods of time.

Four females whose right fallopian tubes were so cooled for 15 minutes were sacrificed at various times after the operation. One killed on the second day after cooling showed a few ovulation points on each ovary, but only one uncleaved ovum was recovered from the cooled tube, none from the left (uncooled side). A second was killed at five days after cooling. Eight ova were recovered from the right oviducts of which one was a collapsed blastocyst, one a morula and the others fragmented or uncleaved. Two others sacrificed at 20 and 21 days, respectively, after the cooling operation



had neither eggs nor embryos, but one had a resorption site in the right uterus.

A number of other animals underwent the cooling operation and were allowed to go to term. The details are given in table 2.

TABLE 2  
THE EFFECTS OF COOLING THE RIGHT FALLOPIAN TUBE CONTAINING FRESHLY OVULATED OVA

NUMBER OF RABBITS	PERIOD OF COOLING (MINUTES)	RESULT
1	5	No young
2	10	No young
7	15	No young
2	20	One gave birth to one living female
4	Frozen with solid CO <sub>2</sub> 2 to 10 minutes	No young

Since these rabbits should have ovulated 12 to 15 ova on the operated side (Pincus<sup>2</sup>) it can be seen that one egg in over 200 developed into a living rabbit. This is less than would be expected if all the ova that presumably cleaved proceeded to develop normally. It has already been shown (Pincus<sup>1</sup>) that rabbit ova artificially activated by other methods may degenerate at any stage of development, and that the expectation of recovery of young is therefore very small.

Full details of these experiments will be published elsewhere.

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<sup>1</sup> Pincus, G., *Jour. Exp. Zool.*, **82**, 85 (1939).

<sup>2</sup> Pincus, G., *Anat. Rec.* (in press).

<sup>3</sup> Shapiro, H., *Science*, **90**, 308 (1939).

*GALACTIC AND EXTRAGALACTIC STUDIES, VI. SUMMARY  
OF A PHOTOMETRIC SURVEY OF 35,500 GALAXIES IN HIGH  
SOUTHERN LATITUDES*

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*1. Introduction.*—The distribution of galaxies on the surface of the sky is easily examined on any uniform collection of long exposure photographs. But an effective study of the distribution in the line of sight requires much greater labor and is complicated by the serious difficulties of nebular photometry and uncertainties concerning the spread of intrinsic luminosity; for when we attempt to measure the space density of external galaxies, and its variation from place to place, it is necessary to use photometric methods for estimating distances. We must measure the apparent magnitudes as dependably as possible, adopt reasonable values of the space absorption and of the mean absolute magnitude and its dispersion, and survey large areas in order to diminish the effects of statistical fluctuations.

It is hoped that through the systematic photometry of large numbers of external galaxies, such as the one summarized in the present paper, it will be possible to trace the metagalactic gradients which have been shown in earlier communications<sup>1</sup> to affect the space density of galaxies within the easily explorable surrounding volume of space that has a radius of the order of a hundred million light years.

A considerable amount of work has already been done at the Harvard Observatory on the frequency of apparent magnitudes and on the immediately related question of space-density variations in the line of sight.<sup>2</sup> The present study, however, has the advantage over some earlier studies of referring only to areas in high latitude. It is not seriously troubled, therefore, by space absorption, unlike the situation in the earlier studies of 36,000 objects in the south celestial polar area (equatorial coördinate system), and of nearly 17,000 in the northern.

Approximately 31,500 galaxies have been measured for the determination of the magnitude frequencies that are summarized in table 2. All the fields lie in galactic latitudes between  $-55^\circ$  and the south galactic pole—a region that appears from examination of the distribution of faint stars on twenty 3-hour exposure, small-scale plates to be wholly free of irregularities in space absorption; it appears in fact, from earlier studies of the south galactic cap, to be entirely free of absorption of the sort that would produce a measurable latitude effect on the magnitudes of external galaxies. From the current study we should obtain, therefore, a very good value for the

mean space-density parameter,\* defined by

$$m_1 = m - \frac{1}{b} \log \bar{N}, \quad (1)$$



FIGURE 1

Frequency of magnitudes on plates *A* 20433 (top), 20503 and 20341. Ordinates are numbers in tenth-of-a-magnitude intervals; abscissae are observed photographic magnitudes.

which relates  $\bar{N}$ , the average number of nebulae (down to a given magnitude) per square degree, to that apparent magnitude  $m$ , on the assumption of uniform distribution (or linear density gradients) throughout space, and

\* The symbol  $N_1$  was inappropriately used for this parameter in the fourth paper of the current series.  $m_1$  is obviously the value of  $m$  to which we must go to find one galaxy per square degree. For uniform space distribution the gradient  $b$  is 0.6.

general similarity in the luminosity function from place to place in the meta-galaxy.

More than twenty individuals have taken part in this investigation, which has been in progress for several years. The marking of the galaxies on the Bruce plates was done chiefly by Mrs. S. F. M. Lindsay, Miss Constance Boyd and Miss Frances Wright. Miss Boyd and Miss Wright have independently estimated the magnitudes. Mr. Robert Porter and Miss Edith Jones made many of the star counts for the establishment of the magnitude sequences. The Bruce plates were made by Dr. J. S. Paraskevo-

TABLE 1  
SUMMARY OF PLATES AND COUNTS

PLATE	RA (1900)	DEC.	$\lambda$	$\beta$	$O$	$m_g$	$m_n$	$N_{tot}$	$N_{25}$	$N_9$
A 20280	22 <sup>h</sup> 48 <sup>m</sup> .8	-10°16'	27.1	-58.4	6	18.4	17.7	1265	1031	340
20318	22 49.9	5 17	35.9	55.4	8	18.3	17.9	2208	1867	729
17182	22 59.3	15 20	23.3	63.4	6	18.6	18.3	797	677	295
15781	23 19.2	0 18	51.6	56.2	5	18.0	17.5	603	546	233
17777	23 40.2	15 13	39.6	71.1	5	18.6	18.5	1343	1177	537
20484	23 59.1	17 38	45.6	76.0	8	19.6	18.4	1641	1441	741
20341	23 59.6	5 23	65.2	65.4	8	18.7	18.3	2495	2078	811
19788	0 18.8	10 17	72.1	71.6	5	18.2	18.0	1383	1138	448
20347	0 18.9	0 31	78.7	61.9	7	18.4	18.2	2892	2457	988
17867	0 19.0	15 16	68.0	76.4	7	18.9	18.8	1838	1601	769
20503	0 38.0	20 20	86.5	82.3	5	18.9	18.2	1656	1538	686
17084	0 39.0	0 20	89.5	62.3	6	18.2	17.7	896	664	308
16253	0 40.0	10 06	90.0	72.1	7	18.5	18.1	1528	1247	588
18691	0 58.9	15 18	110.1	76.6	7	19.3	18.8	4510	3847	1341
16213	1 20.0	10 36	120.3	70.2	6	18.4	18.1	906	740	293
18706	1 38.5	15 15	139.6	71.4	5	18.3	17.6	1021	854	391
18809	1 56.3	20 30	160.8	71.1	6	17.9	17.8	664	575	230
15814	1 58.3	0 14	127.3	56.5	5	18.4	18.0	891	781	299
20440	1 59.3	15 16	149.1	67.7	8	18.4	17.8	1781	1547	796
17946	2 19.3	15 12	156.1	63.7	5	18.1	17.8	1962	1711	839
20433	2 19.5	20 17	166.7	66.3	7	18.2	17.9	2265	1997	905
17971	2 38.0	20 19	170.6	62.1	6	18.9	18.6	2128	1839	951
Means and totals.....					6.2	18.5	18.1	36,673	31,353	13,518

poulos and his assistants at the Boyden Station. Miss Boyd and Miss Martha Dowse have assisted throughout with the calculations and editorial details.

2. *The Observational Material.*—Twenty-two plates, each of three hours' exposure with the Bruce refractor at Bloemfontein, have been used in the present investigation. Because of the general unreliability of faint magnitude standards south of declination  $-23^\circ$ , only plates in the northern part of the south galactic cap were used. In this area both the van Rhijn and the Seares and Joyner tables have been employed in setting up the stellar magnitude sequences. Although the magnitude scale is not as cer-

tain as one could wish fainter than magnitude 17.5, it has the security of being based on the Mount Wilson values of the Selected Area sequences in declinations  $-15^\circ$  and  $0^\circ$ .

In table 1 the twenty-two plates are listed in order of right ascension. The fourth and fifth columns contain the galactic longitudes and latitudes, and the sixth the qualities of the plates (10 represents perfection) from the standpoint of satisfactory discovery and measurement of nebulous objects. The seventh and eighth columns give, respectively, the magnitude  $m_s$  of the faintest stars easily visible on the plates and the magnitude  $m_n$  to which the nebular survey is judged to be complete. The average difference between these two limiting magnitudes, and its mean error, are

$$\overline{m_s} - \overline{m_n} = 0.42 \pm 0.06.$$

The values are similar to those obtained heretofore in similar studies.

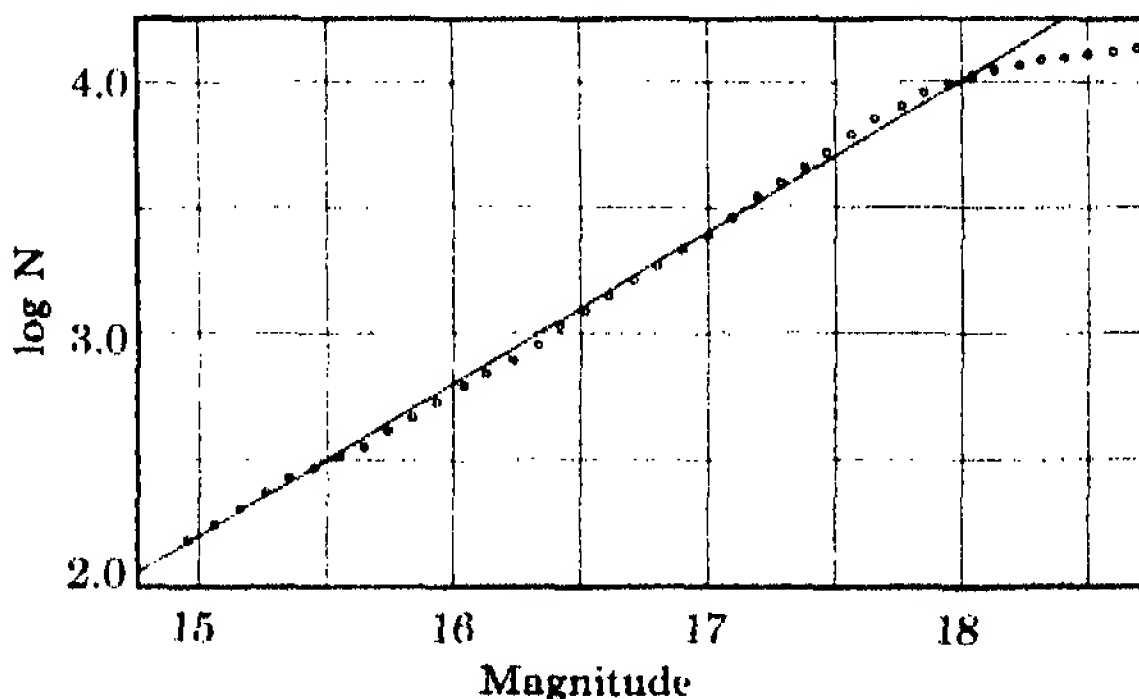


FIGURE 2

Magnitude frequency for all plates. Abscissae are photographic magnitudes corrected for red shift; ordinates are logarithms of the cumulative totals. The straight line is defined by (2).

The last three columns of table 1 give the total number of nebulous objects marked on the plate, the total number in the central twenty-five square degrees, and the total for the central nine square degrees where distance correction can be ignored and there is diminished probability of error of misidentification through deformed images. The grand totals for the whole plate and for the twenty-five square degrees include about twelve hundred and one hundred objects, respectively, that are twice counted through overlapping. Some of these plates appear in an earlier tabulation<sup>3</sup> with totals and magnitude limits differing somewhat from the present values which represent further measurement and analysis.

The magnitudes in intervals of  $0^m.1$  are assembled for each plate in table 2. Although the magnitudes of all objects in the central twenty-five square

TABLE 2  
THE DISTRIBUTION OF MAGNITUDES FOR TWENTY-TWO PLATES

	15.0	15.1	15.2	15.3	15.4	15.5	15.6	15.7	15.8	15.9	16.0	16.1	16.2	16.3	16.4	16.5	16.6	16.7	16.8
A 20280	4	1	0	1	1	3	1	2	1	2	4	1	2	4	1	4	2	3	7
20318	4	0	0	2	3	3	1	2	4	0	3	3	3	3	7	3	8	12	14
17182	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0	1	2	3	4
15781	4	0	0	3	1	5	0	2	1	2	2	2	2	1	0	4	5	5	8
17777	1	0	0	1	0	2	0	2	0	1	0	2	1	1	2	0	5	3	3
20484	3	0	1	1	2	0	1	1	3	2	3	1	4	3	4	8	5	4	4
20341	13	2	2	3	0	2	1	3	5	2	4	3	3	4	4	10	4	11	10
19788	6	3	1	0	1	2	0	1	1	3	2	3	4	3	4	7	4	4	3
20347	2	1	2	1	0	1	3	1	8	4	3	6	5	7	15	23	15	22	25
17867	5	5	1	1	3	2	0	2	7	2	3	3	0	8	4	4	4	6	6
20503	10	1	0	2	0	3	1	3	2	1	1	0	1	0	3	6	4	7	6
17084	7	0	3	3	2	0	1	0	2	2	3	4	10	8	3	6	9	5	9
16253	13	2	2	4	0	1	3	3	5	5	3	7	4	6	6	6	18	10	15
18691	2	0	4	3	2	1	2	2	1	5	6	14	10	9	16	9	11	4	11
16213	2	0	2	3	3	2	0	0	1	2	3	0	2	2	5	2	6	2	9
18706	7	2	2	0	1	3	0	1	6	2	8	9	3	12	8	9	6	13	17
18809	1	0	1	0	0	0	1	0	0	1	0	0	1	2	5	0	7	3	4
15814	3	0	0	1	1	1	0	0	0	1	0	1	4	4	4	6	3	5	6
20440	1	1	0	2	1	0	3	3	0	7	3	2	7	7	10	15	8	14	16
17946	4	2	1	0	0	0	1	2	2	4	2	9	4	7	9	11	9	10	10
20433	17	5	2	4	3	1	2	0	5	2	4	3	9	4	8	11	12	10	20
17971	15	1	2	4	1	1	5	2	6	5	5	6	3	4	8	9	9	14	4
22 Plates	124	26	27	32	34	24	34	28	60	56	62	79	83	99	126	154	156	170	211
13 "	75	15	18	19	18	14	22	17	39	34	33	46	42	51	75	91	90	95	106
5 "	26	6	8	7	9	5	10	8	17	15	17	26	18	25	34	30	34	31	28
Log tot 22	2.09	2.18	2.25	2.31	2.37	2.43	2.47	2.52	2.62	2.67	2.73	2.79	2.84	2.90	2.96	3.03	3.09	3.15	3.21
" " 13	1.88	1.95	2.03	2.09	2.16	2.21	2.24	2.29	2.40	2.46	2.51	2.56	2.61	2.66	2.73	2.80	2.85	2.91	2.96
" " 5	1.41	1.51	1.60	1.69	1.75	1.81	1.85	1.90	2.02	2.08	2.14	2.21	2.26	2.31	2.38	2.43	2.48	2.53	2.56



	16.9	17.0	17.1	17.2	17.3	17.4	17.5	17.6	17.7	17.8	17.9	18.0	18.1	18.2	18.3	18.4	18.5	18.6	18.7	18.7
A 20280	10	19	18	30	21	22	23	30	35	28	25	12	15	6	2					
20318	16	33	19	36	30	27	42	45	74	69	81	66	62	38	16					
17182	4	5	4	8	4	7	12	13	11	20	24	23	21	20	34	29	29	11	1	
15781	6	13	15	30	21	30	27	10	14	6	10	3								
17777	4	14	8	6	17	13	23	18	21	34	43	36	28	47	37	57	66	32	8	
20484	4	11	11	10	20	20	16	20	16	21	26	31	21	44	52	71	74	46	51	125
20341	15	13	10	24	20	29	35	42	46	61	45	35	43	51	63	62	72	43	15	
19788	2	6	7	11	16	21	27	36	42	46	54	61	43	19	3					
20347	19	16	22	27	25	31	48	56	48	60	82	77	103	101	84	38	5			
17867	5	3	8	11	13	10	16	15	18	25	29	31	18	29	28	40	37	60	80	226
20503	9	12	13	14	27	37	33	32	34	44	39	49	35	42	41	40	48	49	32	5
17084	10	10	8	19	19	21	17	21	31	21	21	12	8	9	2					
16253	20	19	23	22	23	26	28	26	30	38	42	49	67	27	19	14	2			
18691	13	11	11	28	21	19	22	35	37	39	30	43	54	66	98	83	101	106	115	295
16213	5	5	4	10	10	8	13	10	21	14	31	31	30	32	16	6	1			
18706	26	15	18	23	26	28	24	32	26	16	17	14	10	2	3					
18809	7	8	13	7	10	17	20	30	22	28	29	13								
15814	1	4	10	11	4	11	9	11	17	42	42	43	32	14	7					
20440	19	15	31	41	51	58	58	69	71	72	65	52	46	28	16	4				
17946	9	10	21	46	45	48	51	63	105	115	106	79	42	10						
20433	16	25	23	21	21	30	39	69	85	94	123	108	79	41	7					
17971	8	11	13	24	38	25	43	34	33	30	51	37	37	49	34	54	79	84	80	81
22 Plates	228	278	310	459	482	538	626	717	837	923	1015	905	794	675	562	498	514	431	382	732
13 "	109	130	144	206	238	257	325	348	374	474	538	546	532	541	516	494	514	431	382	732
5 "	34	50	51	79	109	87	120	122	125	149	179	178	158	235	249	305	357	328	334	727
Log tot 22	3.26	3.33	3.39	3.46	3.53	3.59	3.66	3.72	3.78	3.85	3.90	3.95	3.99	4.02	4.04	4.06	4.08	4.09	4.11	
" " 13	3.01	3.06	3.11	3.18	3.24	3.30	3.37	3.43	3.48	3.55	3.61	3.66	3.71	3.75	3.79	3.83	3.86	3.88	3.90	
" " 5	2.60	2.65	2.70	2.76	2.84	2.89	2.95	3.01	3.06	3.11	3.17	3.22	3.26	3.31	3.36	3.41	3.47	3.52	3.56	

degrees have been measured, only the homogeneous data for the nine central square degrees on each plate are tabulated and used for the present magnitude-frequency discussion. For plate *A* 18691 the nebular counts were made on eight square degrees only, because the ninth contains the rich cluster of galaxies described in these PROCEEDINGS for November, 1939; to correct for the omission, the numbers counted in the eight square degrees have been multiplied by the factor 9/8. At the bottom of the table are totals, sub-totals and logarithms of the cumulative totals and sub-totals, used below in section 3.

The plates are not equally potent; some penetrate to nearly twice the depth of others as indicated by the values of  $m_s$  and  $m_n$  in table 1.

3. *Discussion.*—Table 2 shows the usual diversity in the distribution of magnitudes, illustrated in figure 1 by the data from three plates (central nine square degrees). Similar unevenness is sometimes manifest on a single plate.

Plate *A* 20433 is rich, and down to magnitude 17.5 the frequency fits fairly well the theoretical uniform-density curve (1) for  $m_1 = 15.2$ , which is the average value for the south galactic cap;<sup>4</sup> but apparently a cloud of nebulae is encountered at about magnitude 17.5, doubling the “uniform” number at magnitude 17.8. (A large error in the magnitude scale appearing abruptly at 17.4 would account for the deviation, but is improbable.)

In contrast, plate *A* 20503 is poor, and reaches the “average” curve only at 17.3 to 17.5; it then shows a sub-uniform gradient, the space density falling off sharply with distance until the approaching plate limit disturbs the census at about magnitude 18.5. Plate *A* 20341 is better represented by the lower “uniform” curve, with  $m_1 = 15.5$  and a clustering of about 150 galaxies centered around  $m = 17.6$  at a distance of  $r = 10^{0.2(17.6 + 14.2) - 5} = 23$  megaparsecs, than by the average curve. For all three figures the ordinates are not cumulative totals, but are the numbers for nine square degrees in successive tenth-of-a-magnitude intervals, taken directly from table 2 and not corrected for red shift.

All the data of table 2 are represented by a single logarithmic plot in figure 2. The ordinates are logarithms of the total numbers brighter than the corresponding magnitudes (abscissae). Since  $m_n$  is brighter than 17.7 for two of the plates, the plot cannot be taken as a dependable representation of magnitude frequency fainter than that magnitude. Before making this and subsequent graphs, and all the following calculations, the magnitudes of table 2 were corrected for the red shift.

The straight line that best represents the total material from 198 square degrees, as plotted in figure 2, is given by

$$\begin{aligned} \log N_i &= 0.600m - 6.806 \\ &\pm 0.011 \quad \pm 0.017 \end{aligned} \tag{2}$$

where the mean errors are computed from a least squares solution. From (1) we have

$$m_1 = 15.17 \pm 0.03$$

when we reduce the result (2) to one square degree by the relation

$$\log N_1 - \log 198 = \log \bar{N} = 0.600 (m - m_1).$$

Equation (2) would require, for the area covered by the present survey, eight galaxies brighter than 12.9. An examination of the Shapley-Ames catalog for the central nine square degrees of these twenty-two plates shows three systems, as follows:<sup>5</sup>

NGC 175	12 <sup>m</sup> .8
247	10.7
908	11.1

The relatively small population of bright galaxies in the southern galactic hemisphere has been generally recognized.

The investigation of the magnitude-frequency relation can be carried to fainter magnitudes than involved in (2) by including in the totals and graphs only the data from plates with faint magnitude limits. We thus have in figure 3 two graphs referring to 117 and 45 square degrees, and essentially complete for objects as faint as magnitudes 18.2 and 18.6, respectively. The corresponding linear solutions are given by

$$\begin{aligned} \log N_{117} &= 0.589m - 6.871 \\ &\pm 0.006 \quad \pm 0.009 \end{aligned} \tag{3}$$

$$\begin{aligned} \log N_{45} &= 0.571m - 6.981 \\ &\pm 0.006 \quad \pm 0.012 \end{aligned} \tag{4}$$

which yield the values

$$\begin{aligned} m_1 &= 15.18 \pm 0.015, \\ m_1 &= 15.12 \pm 0.02. \end{aligned}$$

Giving weights 3, 2, 1, respectively, to the three determinations of  $b$  and  $m_1$ , we obtain the mean results

$$\begin{aligned} \bar{b} &= 0.592 \pm 0.009 \text{ (m. e.)}, \\ \bar{m}_1 &= 15.16 \pm 0.02 \text{ (m. e.)}. \end{aligned} \tag{5}$$

Again it is found that there should be eight galaxies brighter than 12.9 in the 198 square degrees.

4. The best representation of the magnitude-frequency plots for individual plates cannot be obtained by linear formulae. The considerable deviations from uniformity in space density, already shown by the plots in

figure 1, and the frequencies in table 2, can be further illustrated by calculating the distribution constants,  $b$  and  $m_1$ , at various right ascensions. It happens that the plates used in computing both the values (3) and (4) are well distributed in right ascension and thus those computations do not provide a test of variation of density across the galactic cap. Assembling the material into three groups of seven plates each,<sup>†</sup> we obtain the following values and mean errors for the radial gradient and the space-density parameter:

	I	II	III
$b$	0.632	0.571	0.611
$\pm$	0.024	0.009	0.020
$m_1$	15.43	15.06	15.09
$\pm$	0.05	0.02	0.05

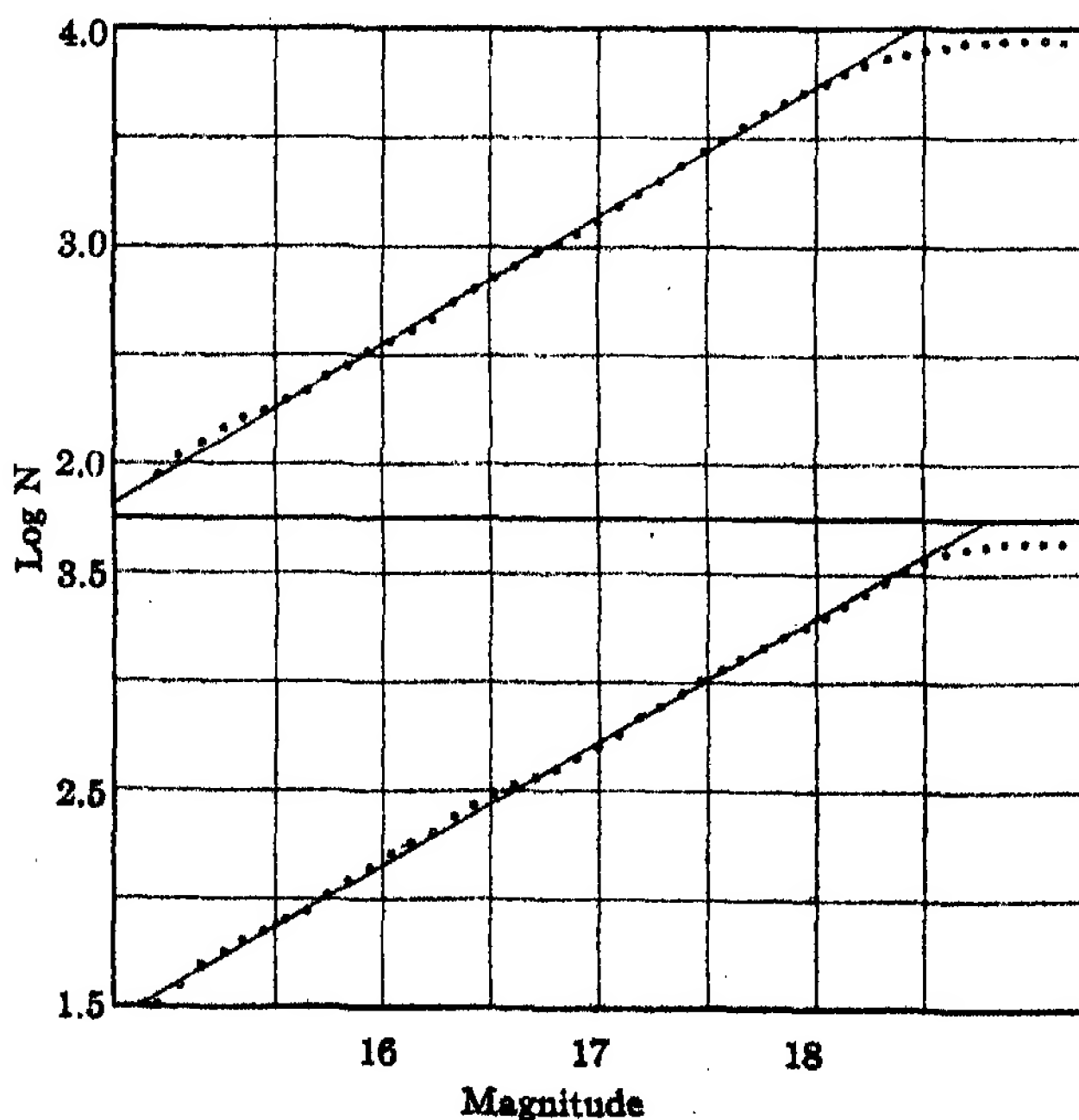


FIGURE 3

Magnitude frequency for thirteen plates (above) and five plates. Coordinates as in figure 2. The straight lines are defined by (3) and (4).

From the measures of  $m_1$  it is seen that the density increases conspicuously with increasing right ascension; a decrease of 0.35 in the parameter corresponds to an increase of about sixty per cent in the space density.

<sup>†</sup> Plate A 18691 is omitted.

In earlier communications<sup>1</sup> attention has been called to the metagalactic gradient that crosses the south galactic cap and extends to the southeastward, nearly to the Milky Way. The plates now under examination lie wholly in the first and second quadrants, but they also show this transverse gradient which appears most conspicuously when first and third quadrants are intercompared.

TABLE 3  
RADIAL GRADIENTS AND SPACE DENSITY PARAMETERS

PLATE	$b$	$m_1$	PLATE	$b$	$m_1$	PLATE	$b$	$m_1$
A 20280	0.67	15.48	20347	0.72	15.24	18706	0.63	15.00
20318	0.76	15.41	17867	0.48	14.88	18809	0.87	16.18
17182	0.78	16.37	20503	0.58	15.16	15814	0.60	15.73
15781	0.63	15.44	17084	0.52	14.90	20440	0.86	15.56
17777	0.75	16.01	16253	0.53	14.62	17946	0.75	15.38
20484	0.62	15.50	18691	0.58	14.92	20433	0.52	14.44
20341	0.57	14.88	16213	0.57	15.41	17971	0.49	14.46
19788	0.57	15.23						

The irregularities in space distribution and possibly in luminosity spread are further shown by a graphical determination of the constants for each plate separately. The results are given in table 3, where the plates are listed in order of right ascension. The gradient  $b$  varies from 0.48 to 0.87;  $m_1$  is systematically fainter for the first part of the table. The mean values and their mean errors are

$$\bar{b} = 0.64 \pm 0.025,$$

$$\bar{m}_1 = 15.28 \pm 0.11.$$

5. *Summary.*—(a) The total photographic magnitudes of something more than thirty-one thousand galaxies have been twice estimated on twenty-two Bruce plates, each of three hours' exposure, on fields in the south galactic polar cap.

(b) The magnitude system for the nebulae is based on that provided for stars through the international standards in Selected Areas; the sequences have been set up by the star-count method.

(c) The high latitude areas covered by this study are free of inequalities in space absorption, if we judge by the distribution of the faint stars; and probably the total dimming of light by interstellar or intergalactic absorption does not here exceed a quarter of a magnitude.

(d) In the coefficients  $b$  of table 3 and in the frequency curves of figure 1 we have numerical and graphical illustration, for this favorably explored region, of the usual deviations from uniformity in the space distribution of galaxies.

(e) In figures 2 and 3 we have what is probably the best information yet

obtained on the magnitude frequency of galaxies in absorption-free non-cluster regions. A good determination of the *average* space-density parameter is therefore possible for this section of intergalactic space. The value  $\bar{m}_1 = 15.16 \pm 0.02$  (m. e.) is derived by a least squares discussion of the assemblages of data used for figures 2 and 3, and  $15.28 \pm 0.11$  (m. e.) from the twenty-two individual values graphically determined for table 3.

(f) Evidence is again found of the strong transverse metagalactic density gradient, across the south galactic cap; but in the line of sight, when all plates are considered together (13,518 objects), the changes of density with distance scarcely exceed the error of measurement. The adopted mean value of the line-of-sight gradient is  $\bar{b} = 0.592 \pm 0.009$  (m. e.).

(g) Taking  $\bar{b} = 0.6$  (uniform space density), we have in the present result an indication of the necessity of the red-shift correction to the photographic magnitudes; of more importance, we have an intimation of the relatively high accuracy of the stellar and nebular magnitude scales from the fifteenth to the eighteenth magnitudes. A systematic error in the scale as large as five per cent is not possible in the mean curves (figures 2 and 3), unless it chances to be almost exactly balanced by an unrevealed radial density gradient.

<sup>1</sup> These PROCEEDINGS, 24, 148, 282, 527 (1938).

<sup>2</sup> Shapley, *Harvard Reprint* 68, 112-115 (1931); these PROCEEDINGS, 21, 589-591 (1935); 23, 450, 452 (1937); and *Harv. Ann.*, 105, No. 8, 146-149 (1937). Seyfert, *Harv. Ann.*, 105, No. 10 (1937). Shapley and Jones, *Ibid.*, 106, No. 1, 5 (1938).

<sup>3</sup> *Harv. Circ.* 423 (1937).

<sup>4</sup> These PROCEEDINGS, 24, 149 (1938).

<sup>5</sup> *Harv. Ann.*, 88, No. 2 (1932).

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## STUDIES IN CALCIUM METABOLISM WITH THE AID OF ITS INDUCED RADIOACTIVE ISOTOPE. I\*

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Communicated February 13, 1940

"Tracer" studies with induced radioactive isotopes have led to important results in the field of mineral metabolism.<sup>1</sup> Calcium is one of the most important of the biological mineral elements. It is one in the study of which a radioactive isotope of calcium as a "tracer" would be very desirable.

The metabolism of calcium is a slow process, and a relatively long time is required for changes to take place. For this reason, the hitherto known radioactive isotope of calcium,<sup>2</sup> with a half life of only about 2.4 hours, has

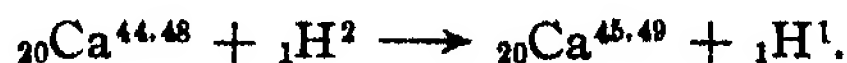


not been suitable. Furthermore, Walke<sup>3</sup> has shown that this isotope, Ca<sup>49</sup> (previously believed to be Ca<sup>45</sup>), disintegrates to form radioactive Sc.<sup>49</sup> Any studies, then, with Ca<sup>49</sup> would be complicated and invalidated by the continuously formed radioactive scandium impurity.

Recently, Walke has reported<sup>3</sup> that Ca<sup>45</sup> is radioactive with a half life of about 180 days. This isotope disintegrates to an inactive scandium with the emission of a very soft  $\beta$ -radiation. The radiation of Ca<sup>45</sup> is so soft that it cannot be measured satisfactorily in metabolism studies with the electroscope, with the FP-54 electrometer,<sup>4</sup> nor with glass or metal-wall Geiger-Müller counter tubes. Highly successful measurements can be made with the screen-wall G-M tube described by Libby.<sup>5, 6</sup>

This communication is a report of a test study on the suitability of the new radioactive calcium isotope for biological investigations. It was found that, with appropriate care, results of great accuracy can be obtained.

*Experimental Methods.*—A sample of radioactive calcium was prepared in the Radiation Laboratory† by the bombardment of calcium metal with 8 m. e. v. deuterons in the cyclotron according to the following nuclear reaction:



After allowing about six weeks to elapse in order that the Ca<sup>49</sup> and Sc<sup>49</sup> might disintegrate to negligible amounts, the metal containing the Ca<sup>45</sup> was dissolved in a dilute HCl solution. Small amounts of inactive NaCl and HCl were added to serve as carriers for the separation of traces of radioactive Na and K. The calcium was precipitated as the oxalate. The precipitate was dissolved in dilute HCl and reprecipitated as the oxalate a second time. The calcium oxalate was then converted to the calcium carbonate by ignition in an electric muffle. This was dissolved in sufficient lactic acid to form a 5 per cent calcium lactate solution. About 8 ml. were obtained.

Five ml. of this 5 per cent calcium lactate solution were given by stomach tube to a male adult rat, weighing 259 grams, and which had been kept without food for 24 hours. Immediately after the administration of the calcium lactate solution, the animal was placed in a wire metabolism cage over a urine-feces separating device.<sup>7</sup> The feces and urine were collected separately at intervals over a period of about 69 hours. A normal diet was supplied ad libitum to the animal 18 hours following the administration of the calcium lactate.

At the end of about 69 hours, the rat was anesthetized with chloroform and sacrificed by withdrawing blood by cardiac puncture. The blood was allowed to clot and the serum was collected. The viscera were removed and the animal was skinned. The contents of the large intestine were washed out and added to the final fecal collection. The residual carcass,

which weighed 156 grams, was boiled in 1:5  $\text{NH}_4\text{OH}$  solution, and allowed to stand in the solution for several days to separate the muscles from the skeleton. The teeth were separated from the bones. The various tissues then were dried and dry ashed at  $500^\circ\text{C}$ . The ash in each case was dissolved in dilute  $\text{HCl}$  solution, and the total solution or an appropriate aliquot was used for analysis. The calcium was precipitated as the oxalate according to the standard analytical procedure. The calcium oxalate precipitate was collected on a 4.25 cm. No. 1 Whatman filter paper, and finally washed with an 0.02–0.03 per cent agar solution to prevent mechanical loss of the precipitate when dry.

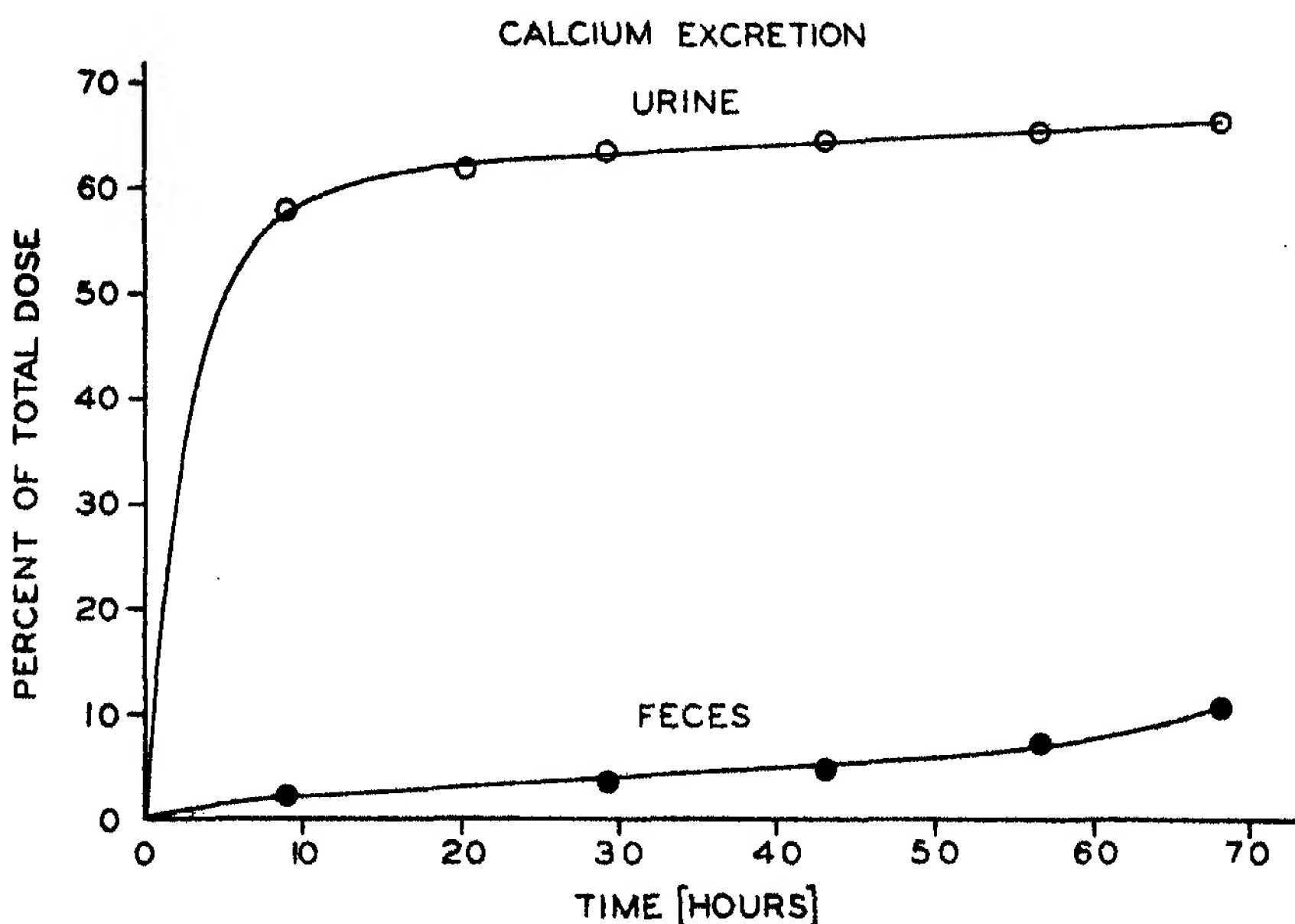


FIGURE 1

Studies in calcium metabolism with the aid of its induced radioactive isotope. I.

The calcium in 0.0976 ml. of the original calcium lactate solution was precipitated and treated in a similar manner to serve as a standard for comparison of activity. All radioactivity measurements were made on the screen-wall counter. The sum of the measured activities of the excreta and of all of the tissues showed a recovery of 103 per cent of the  $\text{Ca}^{45}$  given, so that all figures have been corrected by the factor 100/103.

*Results.*—Figure 1 shows graphically the excretion of  $\text{Ca}^{45}$  during the approximately 69 hours following administration. The amount appearing in the feces was 10.8 per cent. This means that at least 89.2 per cent was absorbed from the alimentary tract. The apparently increasing rate of

excretion in the feces may indicate an excretion of calcium into the intestines. If this were true, the amount absorbed actually would have been a little greater than 89.2 per cent. Of the 89.2 per cent absorbed, 57.9 per cent appeared in the urine during the first 9 hours after administration. The rate of excretion by way of the urine then dropped off rapidly, a total of 65.6 per cent appearing in the urine. This is in harmony with the work of Greenberg and Gunther<sup>8</sup> who showed that certain calcium compounds, including the lactate, caused a marked increase in the diffusible calcium of the blood, which reached a peak two hours after ingestion, and returned to normal within about 4 hours.

TABLE 1  
DISTRIBUTION OF RADIOACTIVE CALCIUM

TISSUES	WEIGHT, GM.		CONTENTS IN WHOLE TISSUE	PER CENT TOTAL DOSE	
	FRESH	DRY		CONTENTS PER GRAM FRESH WT.	CONTENTS PER GRAM DRY WT.
Bones		8.94	19.9 ± 1.76		2.23 ± 0.20
Teeth		0.410	1.13 ± 0.25		2.75 ± 0.61
Blood Serum	5.095	0.358		0.007 ± 0.001	0.094 ± 0.009
Muscle		45.08	0.76 ± 0.066		0.017 ± 0.001
Skin and Hair	51.19	23.88	1.43 ± 0.15	0.028 ± 0.003	0.060 ± 0.006
Stomach	1.86	0.544	0.008 ± 0.004	0.004 ± 0.002	0.015 ± 0.008
Small Intes- tine	5.87	1.65	0.099 ± 0.008	0.017 ± 0.0014	0.060 ± 0.005
Large Intes- tine	1.66	0.62	0.037 ± 0.006	0.022 ± 0.004	0.060 ± 0.010
Liver	9.62	3.06	0.049 ± 0.005	0.005 ± 0.0001	0.017 ± 0.002
Kidney	2.07	0.53	0.007 ± 0.001	0.003 ± 0.0004	0.013 ± 0.002
Spleen	0.79	0.19	0.008 ± 0.003	0.010 ± 0.0037	0.042 ± 0.015
Heart	0.84	0.19	0.017 ± 0.004	0.020 ± 0.0047	0.089 ± 0.021
Lung	0.45	0.27	0.018 ± 0.006	0.040 ± 0.013	0.067 ± 0.022
Testes	5.48	2.71	0.031 ± 0.006	0.008 ± 0.0012	0.012 ± 0.002

The unexcreted  $\text{Ca}^{45}$ , which amounted to 23.6 per cent, was found to be distributed as shown in table 1. As expected, the largest amount of the retained calcium was found in the bones. However, significant amounts were found in the skin and in the teeth. Because of its large mass, the total accumulation of  $\text{Ca}^{45}$  in the skin is considerable, but the specific retention is not so great as in some other tissues. In the case of the teeth, the specific retention of  $\text{Ca}^{45}$  is at least as great as, if not greater than, that in the bones. This would indicate that the calcium of certain parts of the teeth is as mobile as that of the bones.

The concentration of  $\text{Ca}^{45}$  remaining in the serum is small. The specific retention in the small intestine, large intestine, heart and lung is about the same as that in the skin, and is extremely low in the other tissues examined. The small amounts present perhaps are significant, but could be accounted for, at least in part, by occluded blood.

*Summary.*—1. Radioactive  $\text{Ca}^{45}$ , with a half life of 180 days, is suitable for use in "tracer" studies if the radioactivity is measured with a screen-wall counter tube.

2. The radioactivity measurements of a sample of  $\text{Ca}^{45}$ , administered to a rat, showed quantitative recovery in the summation of the measurements on excreta, bone, tooth, skin, carcass, blood and viscera.

3. In a post absorptive state, the rat absorbed at least 89.2 per cent of the calcium given by stomach tube, and excreted 65.6 per cent in the urine.

4. The specific retention of calcium fell off in the following order: highest in bone and teeth, which were about equal; intermediate in small intestine, large intestine, heart, lung and skin; and least in there maining tissues.

We are indebted to Professor E. O. Lawrence, Dr. Harold Walke and the staff of the Radiation Laboratory of the University of California for the radioactive calcium used in this experiment. Our thanks are due also to Professor W. F. Libby for making many of the radioactivity measurements for us with the screen-wall counter, and for allowing us the use of the counter to make the remainder of the measurements.

\* Aided by a grant from the John and Mary R. Markle Foundation. Technical assistance was furnished by the personnel of WPA Official Project No. 65-1-08-62.

† Kindly prepared by Dr. H. Walke, whose recent untimely death lamentably cut short a brilliant scientific career.

<sup>1</sup> Lawrence, J. H., *Handbook of Physical Therapy*, Am. Med. Assoc., 1938; Hevesy, G., *Enzymologia*, 5, 138 (1938), *Jour. Chem. Soc.*, 1213 (1939); Greenberg, D. M., *Ann. Rev. Biochem.*, 8, 269 (1939).

<sup>2</sup> Walke, H., *Phys. Rev.*, 51, 439 (1937).

<sup>3</sup> Walke, H., *Phys. Rev.*, 57, 177 (1940).

<sup>4</sup> Du Bridge, L. A., and Brown, H., *Rev. Sci. Inst.*, 4, 532 (1933).

<sup>5</sup> Libby, W. F., *Phys. Rev.*, 46, 196 (1934).

<sup>6</sup> Libby, W. F., and Lee, D. D., *Ibid.*, 55, 245 (1939).

<sup>7</sup> Gross, L., and Connell, S. V. B., *Jour. Physiol.*, 57, p. 1x (1923).

<sup>8</sup> Greenberg, D. M., and Gunther, L., *Arch. Int. Med.*, 50, 855 (1932).

## *SOME BIOLOGICAL EFFECTS OF NUCLEAR DISINTEGRATION PRODUCTS ON NEOPLASTIC TISSUE\**

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Communicated February 6, 1940

Experiments of the type described below were initiated in the autumn of 1938 by Professors B. V. Hall, M. Goldhaber and the author at the Physics Department of the University of Illinois. Since the neutron intensity from the small cyclotron there was insufficient to give any conclusive result, the experiments have been continued in the Crocker Radiation Laboratory where intense neutron sources are available.

In the past, neoplastic tissue has been irradiated with x-ray,  $\gamma$ -rays and fast neutrons. In the case of x-ray and  $\gamma$ -ray irradiation the destructive ionization in the tissue is produced by Compton electrons, photoelectrons or positive-negative pair electrons which are ejected or created by the x-rays and  $\gamma$ -rays. The physical processes involved here are well known, and the resulting ionization per unit distance along the path of the electron is small as compared to heavy particle ionization.<sup>2</sup>

The process of neutron irradiation is quite different from the above, since it involves a collision process between two heavy particles instead of between a photon and an electron. Here the recoil proton obtains energy (varying from zero to the neutron energy) from the neutron and dissipates the energy by producing along its path an ionization which is much more intense than that produced by electrons.<sup>3</sup>

In the experiments discussed below, the ionizing bodies are the disintegration products produced when boron is bombarded with slow neutrons. In nuclear terms the reaction is represented by  ${}_{5}\text{B}^{10} + n_0^1 \rightarrow {}_{3}\text{Li}^7 + {}_{2}\text{He}^4$ . This reaction is one of the most favorable ones known for use in biological experiments of the type here discussed because the capture cross-section for slow neutrons ( $n_0^1$ ) by boron is about 100 times larger than the collision cross-section for fast neutrons and hydrogen. Thus one would expect this nuclear disintegration process to be more efficient in biology than fast neutron irradiation. Moreover, while the incident slow neutrons have a very small energy (a fraction of an electron volt up to a few electron volts), the disintegration products of the boron slow neutron reaction ( ${}_{3}\text{Li}^7$  and  ${}_{2}\text{He}^4$ ) have approximately 0.8 m. e. v. and 1.4 m. e. v. energy.<sup>3</sup> These rather large nuclear energies are dissipated in very short distances (approximately 4 and 7 microns) in tissue and so cause an even more intense ionization along their paths than the recoil proton in the fast neutron irradiation process. Thus, from the knowledge of nuclear physics alone, it is clear that the boron slow neutron reaction should cause cell destruc-

tion more efficiently than other types of irradiation, if the disintegration can be produced in the environment of neoplastic cells.

This method has the further potential advantage of localizing the lethal ionization in the region where the boron disintegration takes place and thus removes (in the case of its application to *in vivo* work) the danger of

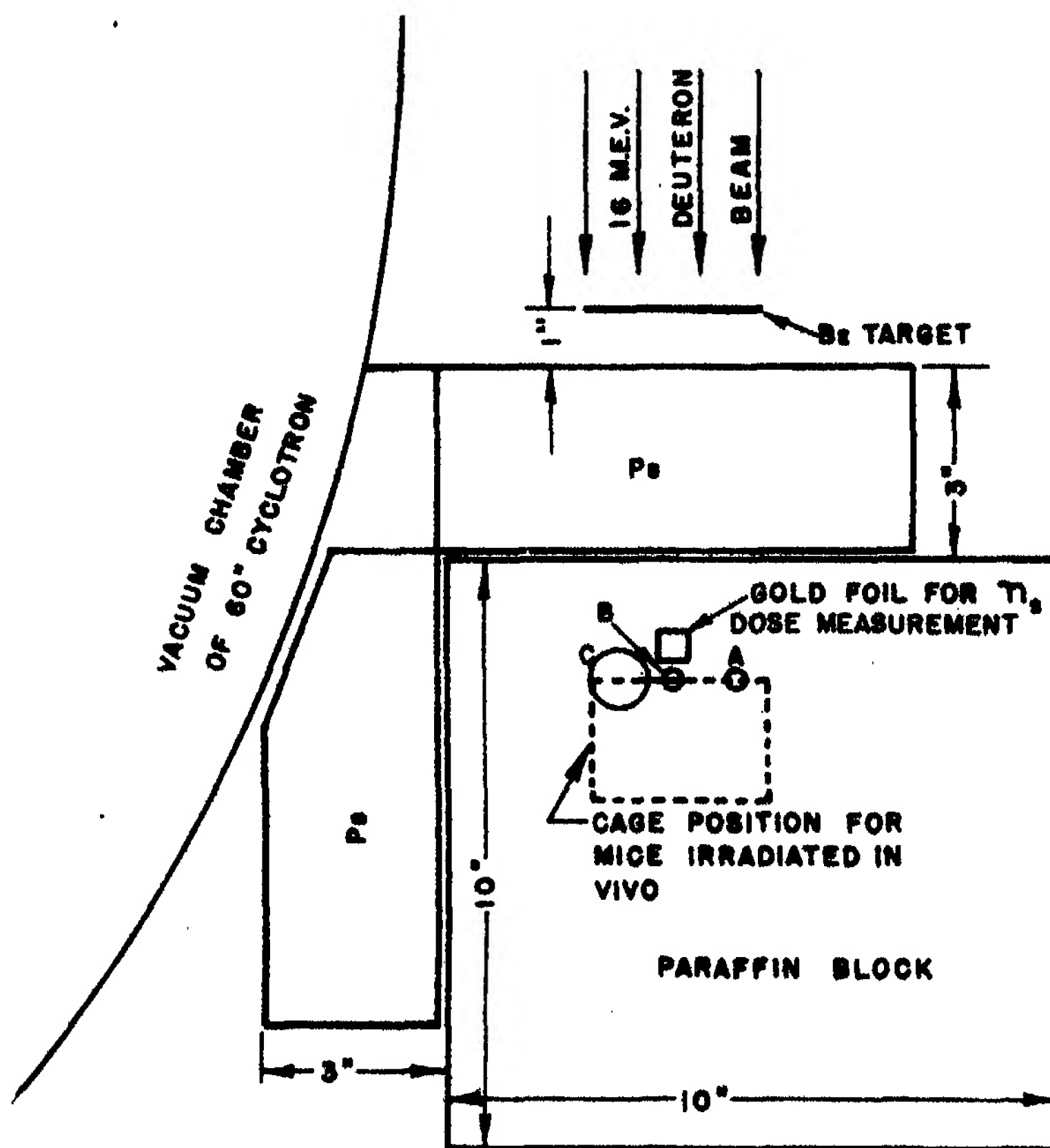


FIGURE 1

Schematic arrangement of beam, target and irradiation positions in the paraffin block.

skin burns and similar disturbing factors which are prevalent in x-ray,  $\gamma$ -ray and fast neutron therapy. This follows from the fact that slow neutrons have so little energy that any ionization caused by a recoil proton from them is negligible. Also it should be remarked that no element having an appreciable concentration in tissue, has a cross-section for slow neutron capture comparable to boron, and thus no ill effects due to slow

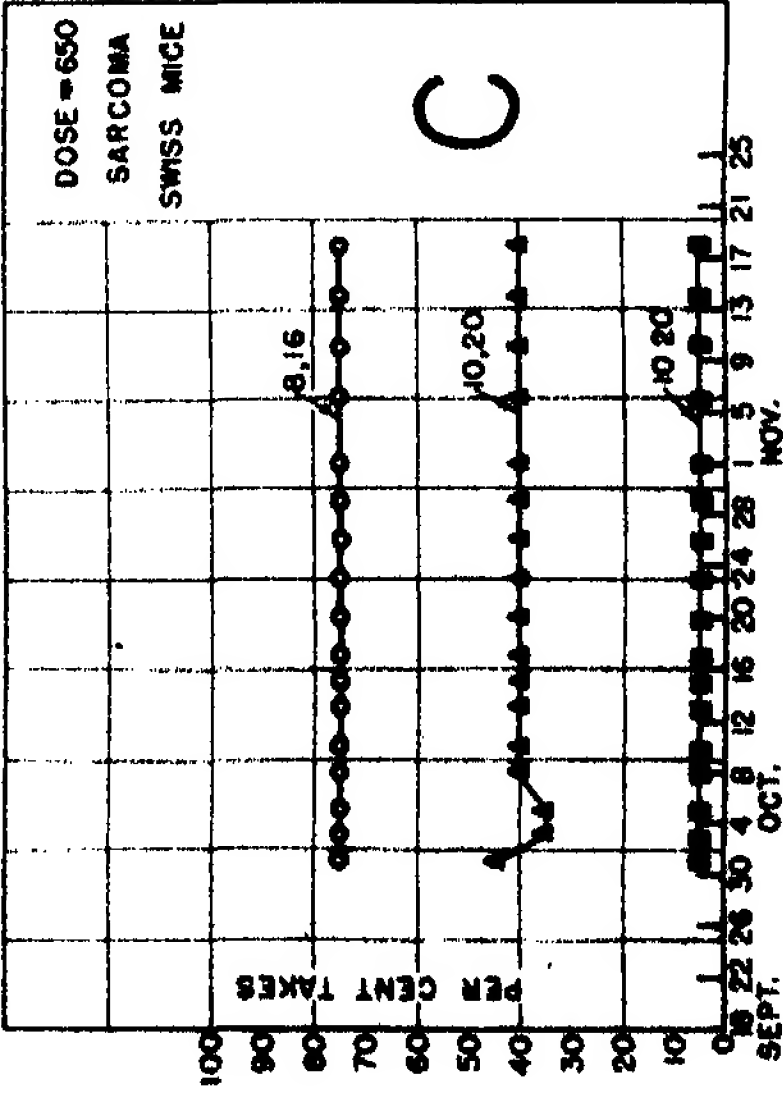
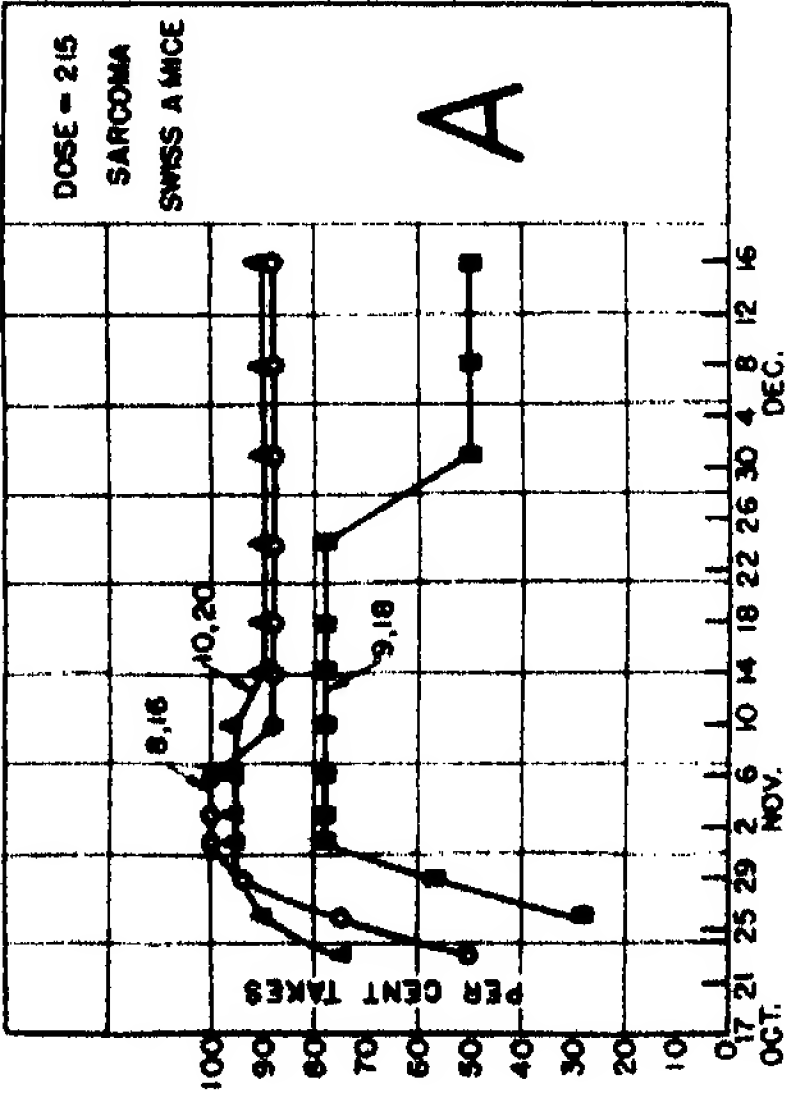
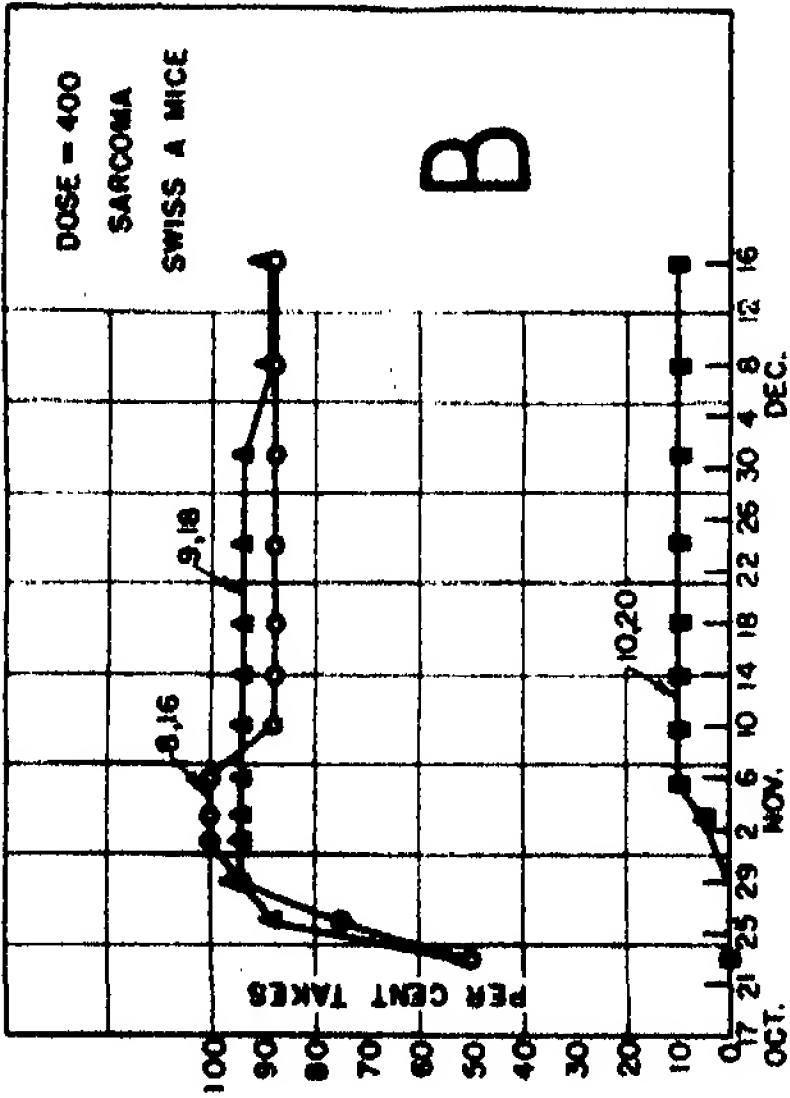


neutron irradiation can occur elsewhere in the body. Consequently one can irradiate with large slow neutron doses, provided care is taken to keep the background dose of fast neutrons and  $\gamma$ -rays below the lethal amount.

Figure 1 shows, schematically, the experimental arrangement for producing the slow neutrons used for the irradiations and the relative positions of the irradiated samples in the paraffin block. Fast neutrons are produced, by bombarding Be with 16 m. e. v. deuterons in the 60-inch cyclotron,<sup>4</sup> according to the reaction  ${}^9_4\text{Be} + {}^2_1\text{D} \rightarrow {}^{10}_5\text{B} + {}^1_0\text{n}$ . These fast neutrons are slowed down by many collisions with hydrogen nuclei in the paraffin block and are thus available at positions *A*, *B* and *C* for irradiating samples placed at *A*, *B* or *C*. The Pb blocks shown in figure 1 were placed between the target, cyclotron and the irradiated samples to reduce the  $\gamma$ -ray background from the Be target. A thin sheet of gold (Au detector in figure 1) was positioned just in front of hole *B* in the paraffin block and the slow neutron induced radioactivity in the gold used as a measurement of the slow neutron dose for the various irradiations. Measurements of the gold activity were made in the conventional manner using an ionization chamber, amplifier and a scale of four counter. The fast neutron- $\gamma$ -ray background was measured with a victoreen dosimeter.

The procedure for preparing small pieces of mammary carcinoma, lymphoma and an undifferentiated sarcoma for irradiation and implantation is as follows. A tumor, about ten days old, is taken from the animal and chopped into small pieces suitable for implantation with a trocar. These are placed in a soft glass test tube about  $\frac{1}{4}$  inch in diameter and immersed in a solution made by adding 2 gm.  $\text{H}_3\text{BO}_3$  to 100 cc. of buffer solution. Three such samples are made up. One is kept in the laboratory as a control and is not irradiated. This hereafter will be designated as the boron control. A second, the boron irradiated, is placed in position *A* in the paraffin block. The third is placed inside of a one-inch diameter glass tube and the intervening space filled with  $\text{B}_6\text{C}$ . This is placed in position *C* and is designated as a *B* + *B* shield. A fourth sample has been prepared for some experiments (mammary carcinoma *A*, *B*, *E* and lymphoma *C*) by omitting the  $\text{H}_3\text{BO}_3$  from the immersing solution. This is placed in hole *B* and is called the buffer control. All tumors used were known by previous experimentation to give essentially 100 per cent takes for normal implants.

During the course of an irradiation the boron irradiated sample (*A*) receives  $\gamma$ -rays ( $\gamma$ ) and fast neutrons ( $n_f$ ) as background radiation and slow neutrons ( $n_s$ ). Sample *C* receives mostly background  $n_f + \gamma$  radiation, the  $\text{B}_6\text{C}$  absorbing a large part of the slow neutrons except for heavy doses. Thus the resultant differential growth between samples *A* and *C* represents, in a rough way, the effect of the slow neutrons. Sample *B* receives  $n_f + \gamma + n_s$  and is simply a control to test for any possible effect



- B CONTROLS
- △ B + B SHIELD IRRADIATED
- B IRRADIATED

FIGURE 2

Survival curves for an undifferentiated sarcoma irradiated, *in vitro*, with the disintegration products from the reaction  $B^{10} + n_0^1 \rightarrow Li^7 + He^4$ . Per cent takes are plotted against the number of days after implantation. The first date on the x-axis gives the time of irradiation and implantation.

of irradiation in buffer solution. None was observed as would be expected since none of the elements in the buffer solution have a slow neutron capture cross-section comparable with boron.

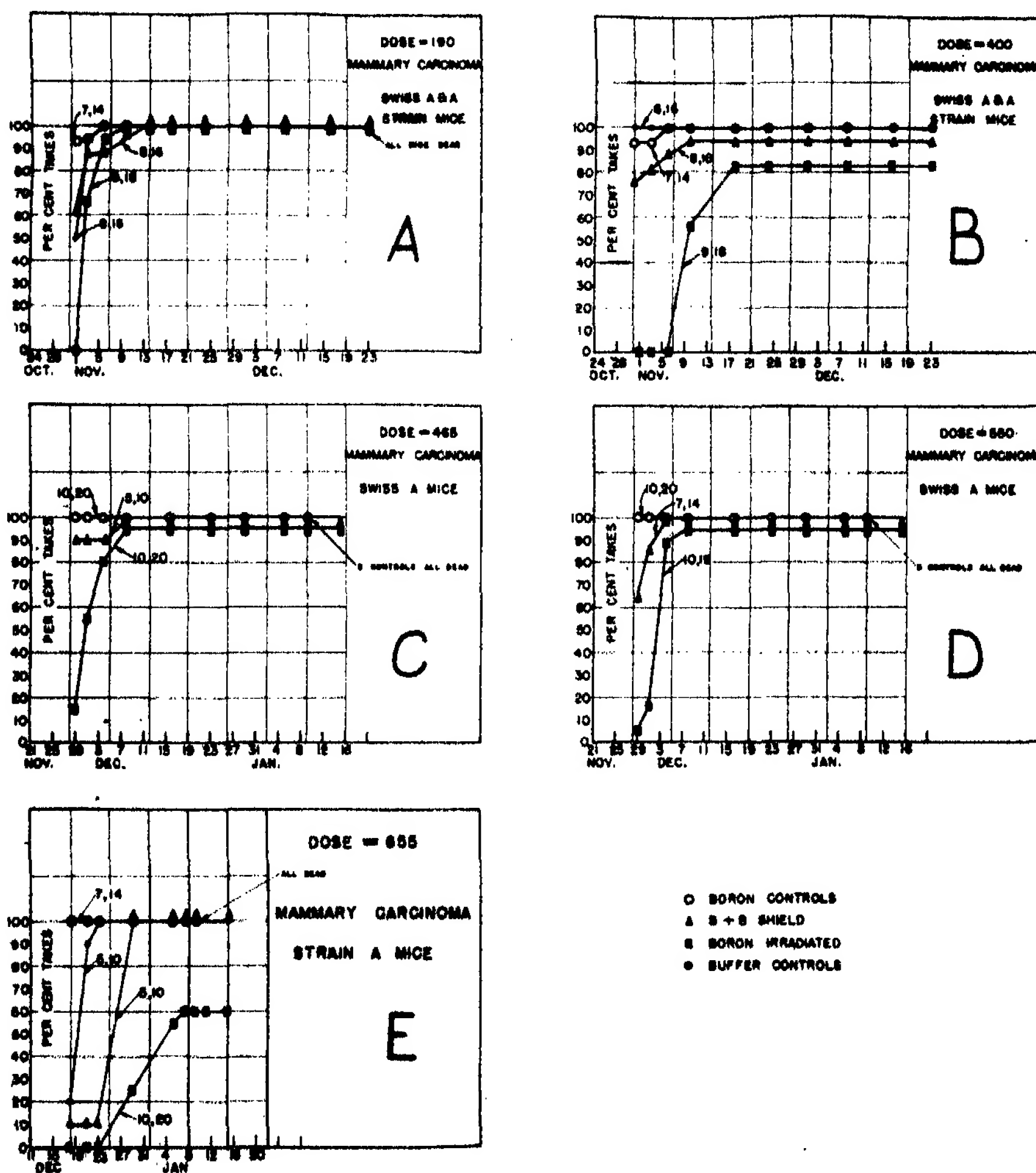


FIGURE 3

Survival curves for mammary carcinoma irradiated, *in vitro*, with the disintegration products from the reaction  ${}_{5}\text{B}^{10} + n_0^1 \rightarrow {}_{3}\text{Li}^7 + {}_{2}\text{He}^4$ . Per cent takes are plotted against the number of days after implantation.

After irradiation, two tumor particles are implanted in each mouse (one on each side) used. Thereafter for about eight weeks, the number of takes, for each experiment, was checked at least once a week by counting the number of tumors observable in each mouse and by measuring the

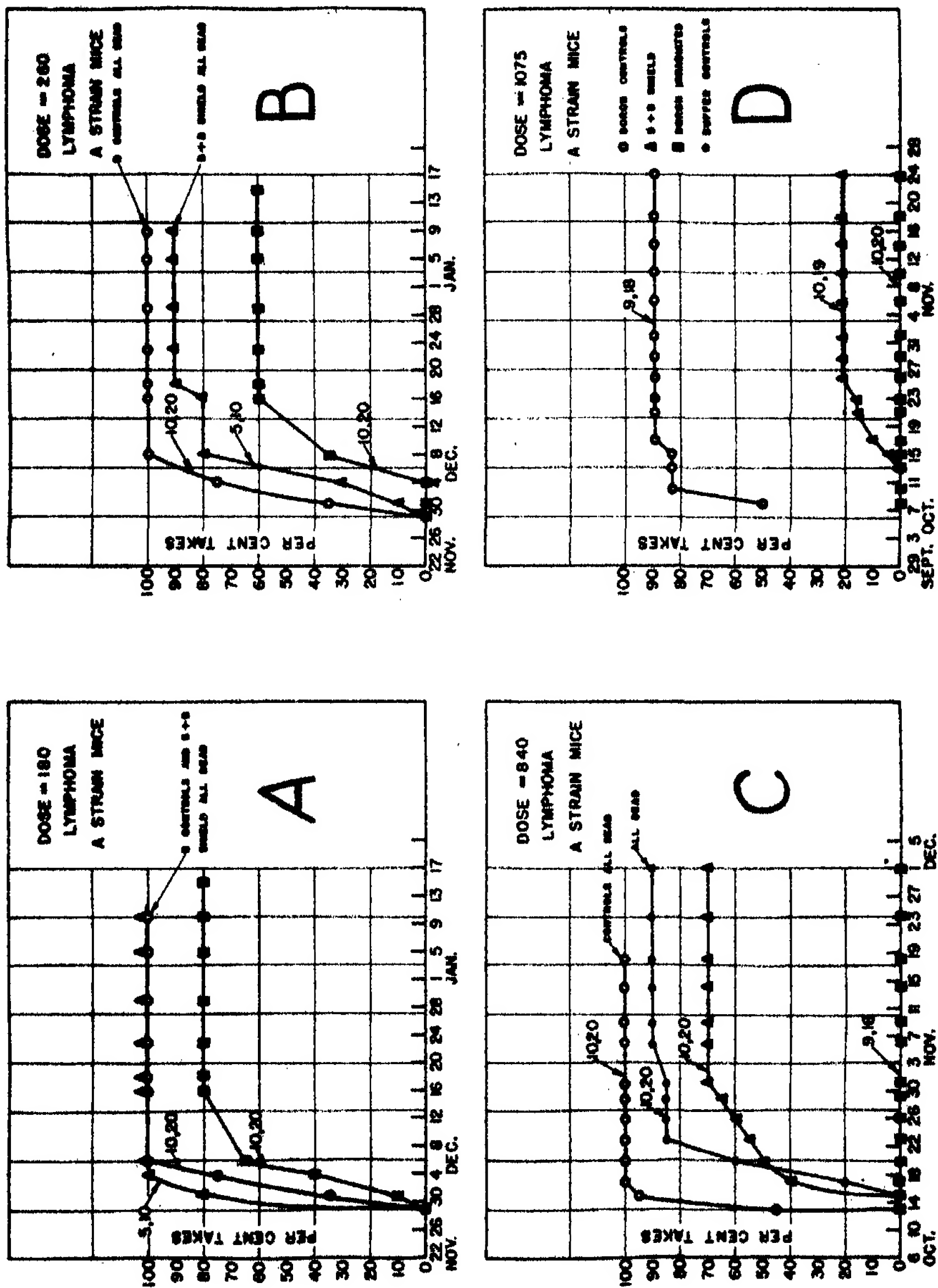


FIGURE 4

Survival curves for lymphoma. Other conditions the same as those of figures 2 and 3.

size of the tumors. The results of these measurements are shown graphically in figures 2, 3 and 4.

Figure 2 shows the effect of the boron disintegration products on an undifferentiated sarcoma which occurred spontaneously in a swiss mouse four years ago, and since then has been observed and studied by Professor B. V. Hall of the University of Illinois. The figure has three sets of curves (*A*, *B* and *C*), one for each of three doses given the tumor particles prepared and irradiated as described above. The dose, given in the upper

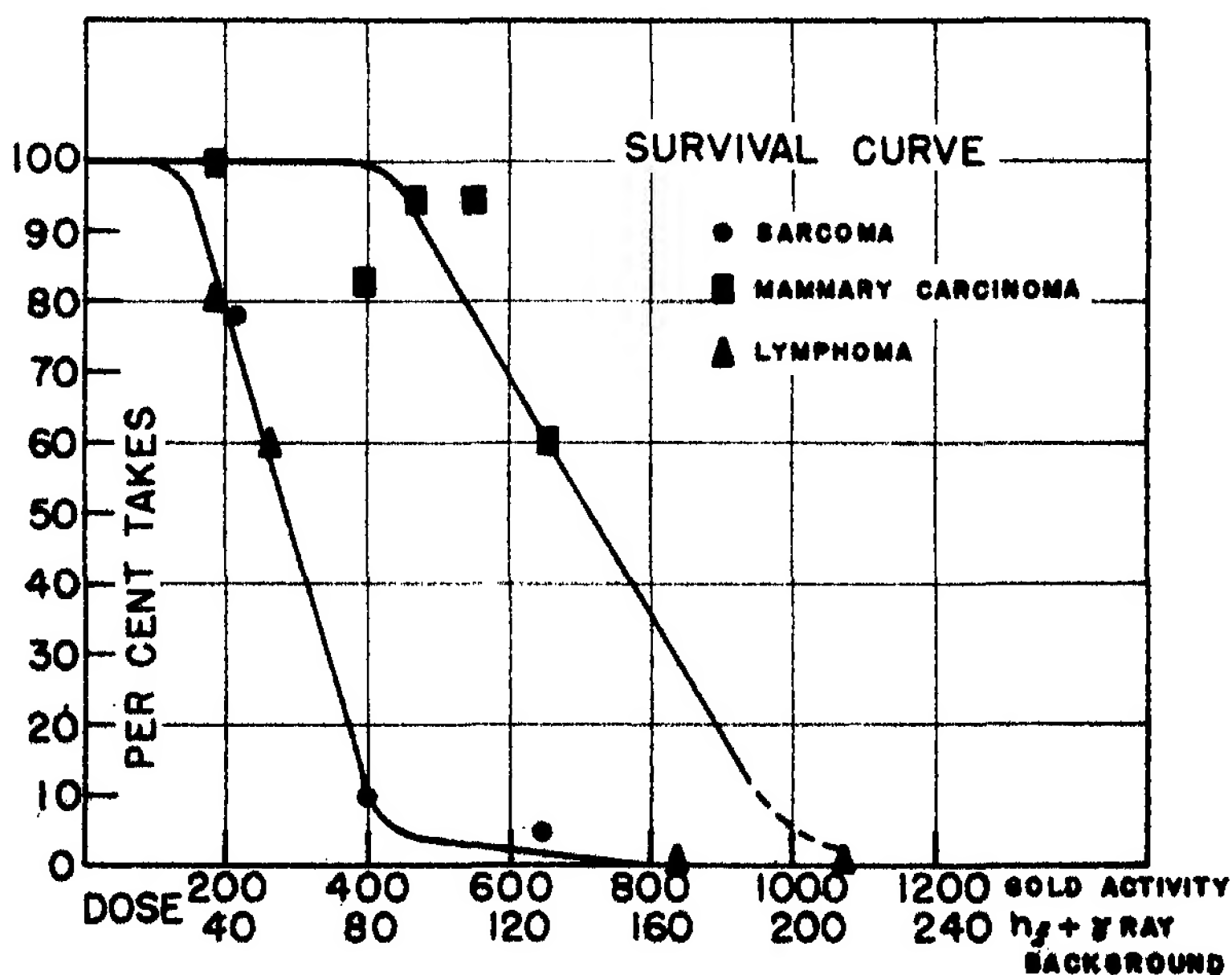


FIGURE 5

Comparison survival curves for sarcoma, mammary carcinoma and lymphoma. Here the maximum per cent number of takes are plotted against the slow neutron dose as measured by gold activity and for reference on another scale the associated  $n_t + \gamma$  background dose.

right-hand corner of parts *A*, *B* and *C* of the figure, is the result of the measurement of the radioactivity induced in the gold foil (see Fig. 1) by slow neutrons during the tumor irradiation. For this reason it is a relative dose measurement and, while comparable for all of the experiments described here, has no direct comparison with other dose measurements (i.e., fast neutron doses as measured by a victoreen dosimeter) or even other gold activity dose measurements made under different experimental conditions. Associated with each curve are two numbers, the first one of which gives the number of mice used, the second the number of tumors

implanted (i.e., curve in figure 2A for boron irradiated samples has the numbers 9, 18: this means 9 mice and 18 implants).

In a qualitative way the boron shield acts as would be expected. For doses 215, 400 and 650 (Fig. 2A, B, C) the per cent takes are 95, 94 and 40 per cent, respectively. This indicates that for the first two doses enough slow neutrons were absorbed by the  $B_6C$  shield so that the transmitted neutrons had little effect on the tumors. However, at dose 650 enough slow neutrons were transmitted to cause 60 per cent deaths. For the

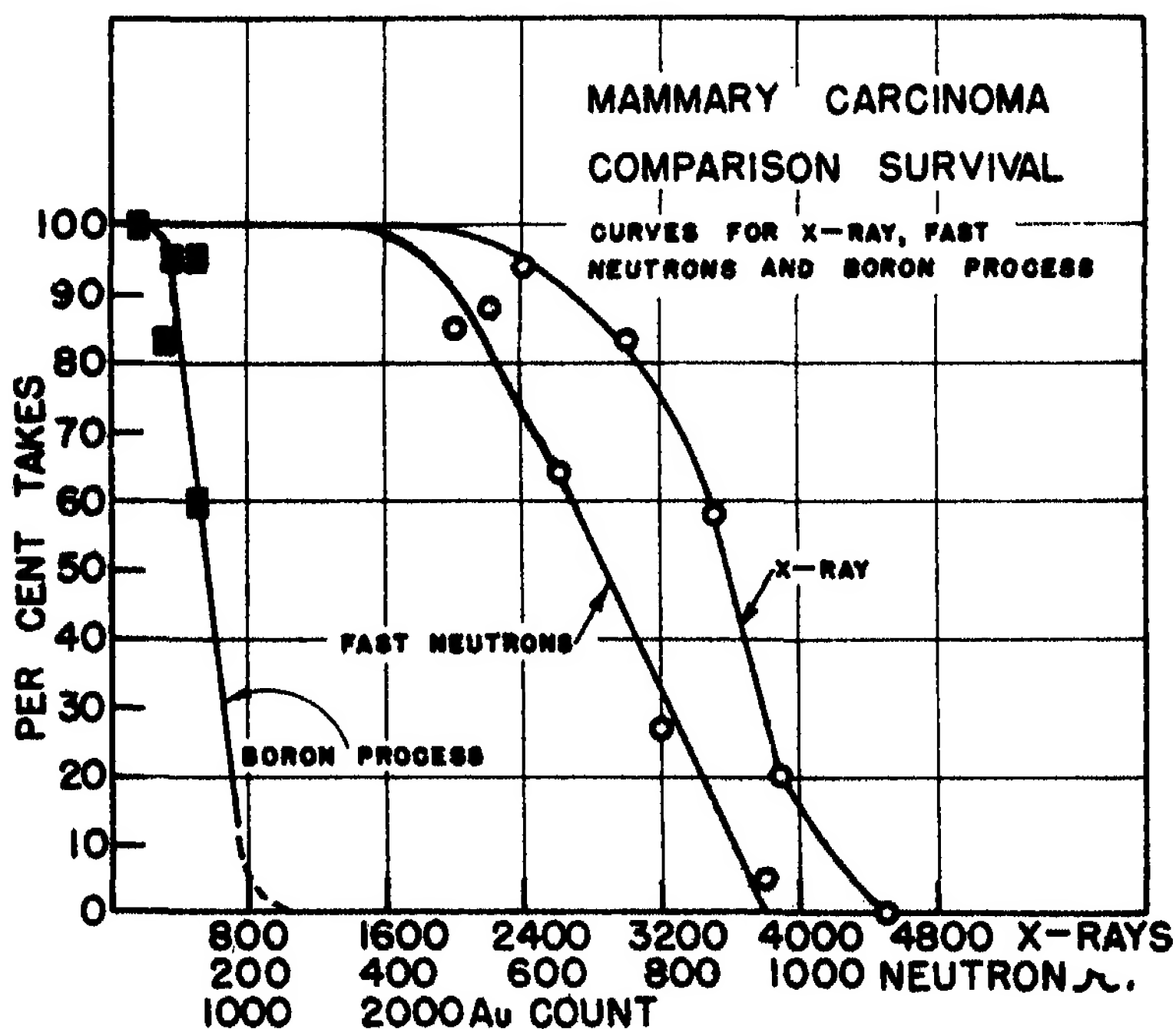


FIGURE 6

Comparison survival curves for mammary carcinoma irradiated with x-ray, fast neutrons and boron disintegration products.

above doses the  $n_f + \gamma$  background was approximately 45, 80 and 130 "n." No data concerning the effect of fast neutrons on this sarcoma are available.

Figure 3 shows the effect of the boron disintegration products on mammary carcinoma.<sup>5</sup> The notation here is the same as that for figure 2. In three (A, B and E) of the five experiments performed on this tumor a group of animals were inoculated with implants irradiated in buffer solution as described above. All three groups show 100 per cent takes as was expected from theoretical considerations. The only other similar experi-



ment performed was in part C of the lymphoma experiments where 90 per cent takes were observed.

Figure 4 shows the effect of the boron disintegration products on lymphoma.<sup>6</sup> The notation is the same as for figure 2. Here the effect of the boron shield is nicely portrayed. For the doses 180, 260, 840, 1075, the per cent takes for the boron shield irradiated implants are 100, 90, 70 and 21 per cent. The  $n_t + \gamma$  background corresponding to the above doses is 35, 50, 170 and 215 "n."

The data in figures 2, 3 and 4 are given in tabular form in table 1.

TABLE 1  
SUMMARY OF DATA ON THE *in vitro* IRRADIATION OF SARCOMA, MAMMARY CARCINOMA AND LYMPHOMA WITH DISINTEGRATION PRODUCTS FROM THE REACTION  ${}_5\text{B}^{10} + {}_1\text{n}^1 \rightarrow {}_3\text{Li}^7 + {}_2\text{He}^4$

Sarcoma					
DOSE FROM GOLD ACTIVITY	APPROXIMATE BACKGROUND $n_t + \gamma$	PER CENT TAKES BORON IRRADIATED	PER CENT TAKES B + B SHIELD	PER CENT TAKES BORON CONTROLS	PER CENT TAKES BUFFER CONTROLS
215	45	78	95	100	
400	80	10	94	100	
650	130	5	40	75	
Mammary Carcinoma					
190	40	100	100	100	100
400	80	83	94	100	100
465	90	95	100	100	
550	110	95	100	100	
655	130	60	100	100	100
Lymphoma					
180	35	80	100	100	
260	50	60	90	100	
840	170	0	70	100	90
1075	215	0	21	80	

In figure 5 there is plotted the maximum per cent number of takes (*B* irradiated sample) taken from the curves in figures 2, 3 and 4, vs. the dose for the three tumors used. Here it appears that the sarcoma and lymphoma have about the same sensitivity to the radiation used and that a dose of 450 (gold activity) will kill both kinds of tumors *in vitro*. This corresponds to a  $n_t + \gamma$  background of about 90 "n." The mammary carcinoma is more resistant to radiation and needs a dose of about 1000 (gold count) with a background of 200 "n" for 100 per cent lethal effects.

Figure 6 shows a comparison between the effects of x-rays, fast neutrons and boron disintegration products on mammary carcinoma. The x-ray and fast neutron data are taken from curves published by J. H. Lawrence, P. C. Aebersold and E. O. Lawrence.<sup>2</sup>

The fast neutron curve shows that below approximately 500 "n," no failure of takes occurs. Since, for the boron process, the  $n_i$  background accompanying the lethal dose (gold activity 1000) of boron disintegration products is only 200 "n," that background cannot be responsible for the lethal effects observed, and one must conclude that the boron disintegration products are responsible for the death of the tumor cells. Another

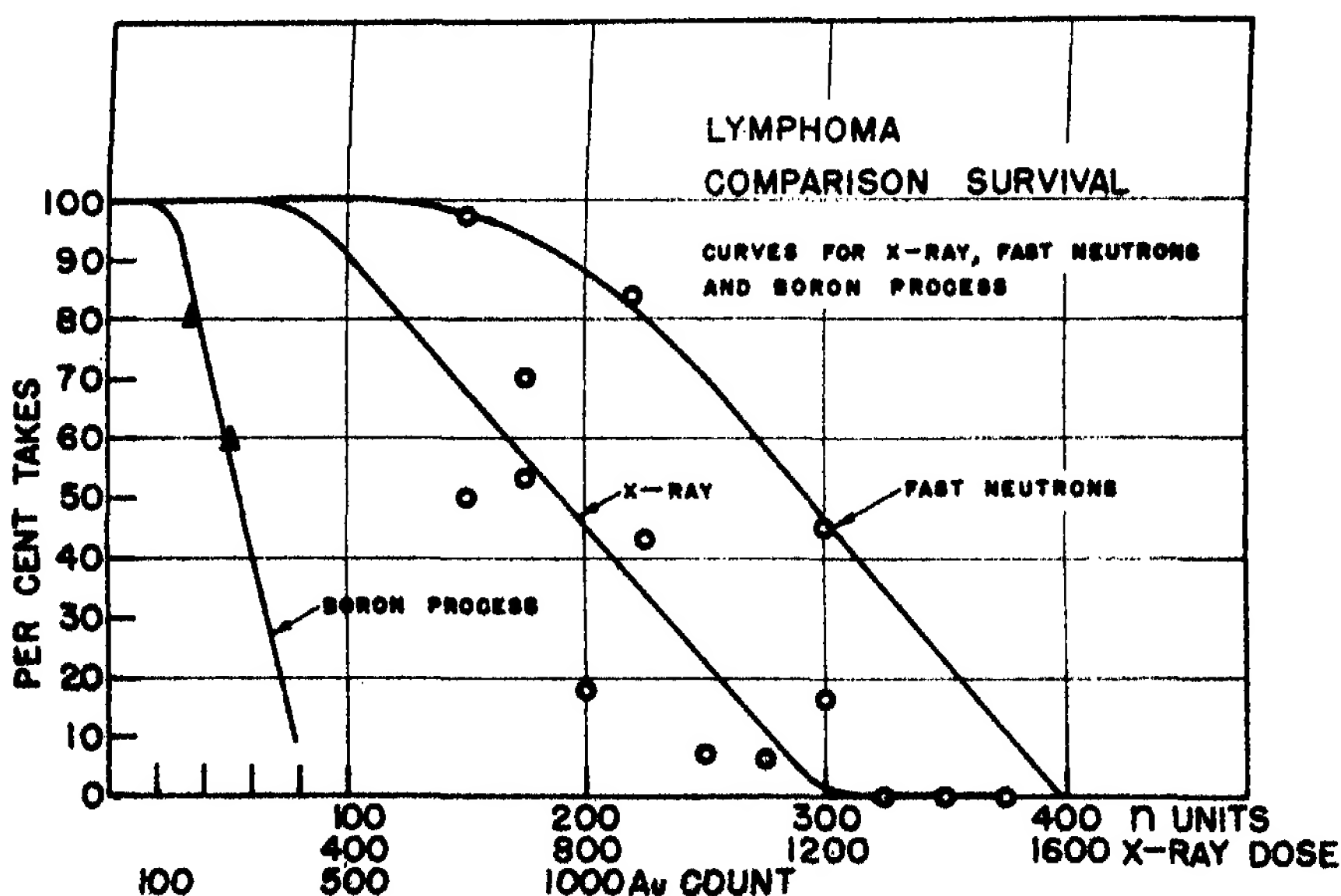


FIGURE 7

Comparison survival curves for lymphoma irradiated with x-rays, fast neutrons and boron disintegration products.

interesting comparison is to note that the dose of fast neutrons for 100 per cent lethal effects is approximately 950 "n," which is about five times the  $n_i$  background in the boron process for the same effect. It must be emphasized, however, that the factor five depends on the amount of boron which can be gotten into the tumor and that the factor is meaningless except for the fact that it shows that a sufficient amount of boron can be dispersed throughout the tissue, to accomplish the desired lethal effect.

Figure 7 draws a similar comparison for lymphoma. Here the fast neutron data are taken from preliminary experiments being conducted<sup>7</sup> in the Crocker Radiation Laboratory at the present time and the x-ray

data from exploratory and unconfirmed results. The fast neutron sub-lethal dose is approximately 175 "n" whereas the  $n_\gamma + \gamma$  background accompanying 100 per cent lethal effect in the boron process is about 90 "n" so that again the lethal effects observed here must be due to the boron disintegration products. The fast neutron dose for 100 per cent lethal effect is approximately 400 which is about four times the  $n_\gamma + \gamma$  background in the boron process for the same effect.

As shown by the data in table 1, the average per cent number of takes for the boron controls in the three experiments on sarcoma is 92 per cent; for the five experiments on mammary carcinoma it is 100 per cent; and for the four experiments on lymphoma it is 97 per cent. This shows that the boric acid solution when not irradiated has no effect on the growth of these neoplastic tissues.

In considering the data from these experiments, it must be remembered that the number of mice used (see figures 2, 3 and 4) was small so that the shape of the survival curves in figure 5 is known only approximately. It would be of interest to repeat these experiments with a large number of mice to establish the curves more accurately.

In figure 2A and B there is evidence for some natural regression of the undifferentiated sarcoma. In those cases where regression occurred the tumors grew to good size (1 cc. to 2 cc. volume approximately), became necrotic and then sloughed off. Eventually some healed completely so that it is unsafe to use this tumor for *in vivo* work.

The results of these *in vitro* experiments also indicate that neoplastic cells can be destroyed *in vivo*, if sufficient boron, in some suitable form, can be applied to the tumor *in vivo*.

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It is a pleasure for the author to express his thanks to Professor E. O. Lawrence for the opportunity of working in the Crocker Radiation Laboratory, and to Dr. J. H. Lawrence for his many helpful suggestions and interest in the work.

He also wishes to thank Miss D. Axelrod and Miss V. Condit for their help in preparing and making the tumor implants.

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<sup>1</sup> On sabbatical leave from the Physics Department, University of Illinois, Urbana, Illinois.

<sup>2</sup> J. H. Lawrence, P. C. Aebersold and E. O. Lawrence, Occasional Publications, Am. Assoc. Adv. Sci. No. 4, 215-219 (June, 1937).

<sup>3</sup> Bower, Bretcher and Gilbert, *Proc. Camb. Phil. Soc.*, 34, 290 (1938).

<sup>4</sup> Lawrence, Alvarez, Brobeck, Cooksey, Corson, McMillan, Salisbury and Thornton, *Phys. Rev.*, 56, 124 (1939).

<sup>5</sup> W. U. Gardner, G. M. Smith, E. Allen and L. C. Strong, *Arch. Path.*, 21, 265

(1936); J. H. Lawrence, Robert Horn and L. C. Strong, *Yale Jour. Biol. and Med.*, 10, 145 (1937).

<sup>6</sup> J. W. Lawrence and W. U. Gardner, *Amer. Jour. Cancer*, 33, No. 1, 112 (May, 1938).

<sup>7</sup> The experiments are being conducted by J. H. Lawrence, P. C. Aebersold and D. Axelrod.

## A DECOMPOSITION OF COMPACT CONTINUA AND RELATED THEOREMS ON FIXED SETS UNDER CONTINUOUS TRANSFORMATIONS<sup>1</sup>

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Communicated January 30, 1940

1. *Definitions and Theorems on F-sets.*—We suppose throughout that  $M$  is a compact metric continuum.

DEFINITIONS:<sup>2</sup> A point  $p \in M$  is *conjugate* to  $q \in M$  provided no point separates  $p$  and  $q$  in  $M$ . If  $p$  is a non-cut point,  $Mp$  is defined to be the set of all points conjugate to  $p$ .  $p \in M$  is an *end-point* of  $M$  provided there exists an arbitrarily small neighborhood of  $p$  having as its boundary a single point. A set is said to be an *F-set* provided it is (1) an end-point of  $M$ , (2) a cut point of  $M$  or (3) a non-degenerate  $Mp$ .

THEOREM 1.1: Any set  $Mp$  may be written as a monotone product  $Mp = \prod_{i=1}^{\infty} C_i$ , where each  $C_i$  is a continuum, the closure of the complement of which consists of a finite number of continua, each intersecting  $C_i$  in a single point.

This theorem is proved by a direct construction, making use of the lemma to the effect that there exists a countable basis for the cut points of  $M$ , i.e., a countable set of points  $[p_i]$  such that if any two points of  $M$  are not conjugate, some point of  $[p_i]$  separates them in  $M$ . This fundamental theorem implies

THEOREM 1.2: An *F-set* is a continuum: the product of an *F-set* and a continuum is a continuum or vacuous.

THEOREM 1.3:  $M$  is the sum of its *F-sets*.

This theorem is proved by showing that if  $p \in M$  is not an end-point and has no conjugate point, it is a cut point. This gives an independent proof of the known result when  $M$  is locally connected. For non-locally connected continua the result is new. From this theorem we obtain

THEOREM 1.4: Each non-cut point of  $M$  belongs to one and only one *F-set*.

THEOREM 1.5: In order that two points  $p$  and  $q$  belong to the same *F-set* it is necessary and sufficient that  $p$  and  $q$  be conjugate.

If  $p$  and  $q$  are conjugate we obtain a non-cut point conjugate to both, and from this an  $F$ -set, by use of the known result that there exists no uncountable collection of mutually conjugate cut points.

As a consequence of 1.1 and the preceding we get

**THEOREM 1.6:** *If a point  $p$  does not belong to a true  $F$ -set (i.e., an  $F$ -set containing more than one point), it is a regular point in the sense of Menger-Urysohn.<sup>3</sup>*

Also from 1.5 we obtain

**THEOREM 1.7:** *The product of two  $F$ -sets is either a cut point or vacuous. Also, there are in  $M$  only a countable number of cut points belonging to true  $F$ -sets.*

The proof of the second of these statements requires also the cut point order theorem.<sup>4</sup>

**THEOREM 1.8:** *In order that a non-degenerate subset of  $M$  be a true  $F$ -set it is necessary and sufficient that it be separated in  $M$  by no point of  $M$ , and that it be saturated in  $M$  relative to this property.*

This theorem is established on the basis of 1.5.

2.  $J$ -sets:  $F$ -set reducible and extensible properties.

**DEFINITION:** A subcontinuum of  $M$  is a  $J$ -set if it is the sum of  $F$ -sets.

This is the precise analogue of the  $A$ -sets in the cyclic element theory for locally connected continua. We show from 1.2 and 1.5

**THEOREM 2.1:** *The product of any continuum  $D$  with a  $J$ -set  $J$  is connected.*

From 2.1, we obtain

**THEOREM 2.2:** *The product of any number of  $J$ -sets is either a  $J$ -set or vacuous. A  $J$ -set  $J$  contains all the irreducible continua about any two of its points.*

Analogous to cyclicly extensible and reducible properties we make the following

**DEFINITION:** A property is  $F$ -set reducible provided that when  $M$  has the property, so also has every  $F$ -set in  $M$ . A property is  $F$ -set extensible provided that when every  $F$ -set in  $M$  has the property, so also has  $M$ .

**THEOREM 2.3:** *Unicoherence is  $F$ -set extensible (but not in general  $F$ -set reducible).*

If  $M$  is locally connected it is known that unicoherence is both cyclicly extensible and reducible.

An example is given showing that the fixed point property is not in general  $F$ -set reducible, and that an  $F$ -set is not necessarily a retract of the space  $M$ .

3. Transformations of a compact continuum into itself.

**THEOREM 3.1:** *If  $T(M) \subset M$  is a continuous transformation of a compact continuum  $M$  there exists a continuum  $\pi \subset M$ , which is a subset of some  $F$ -set of  $M$ , such that  $T(\pi) \supset \pi$ .*

Given  $T(M)$ , it is shown that there exists a continuum  $N$ , irreducible

with respect to the property of being the product of continua of type  $P$ , where a continuum  $C$  is said to be of type  $P$  provided the closure of the complement of  $C$  consists of a finite number of components  $D_i$ , each  $D_i$  intersecting  $C$  in a single point  $p_i$ , and where  $T(p_i) \bar{\in} (D_i - p_i)$ . It is then shown that  $N$  is a subset of an  $F$ -set, and finally that  $N$  contains a continuum  $\pi$  such that  $T(\pi) \supset \pi$ .

As results of 3.1 we obtain

**THEOREM 3.2:** *If  $T(M) \subset M$  is a continuous transformation of a compact continuum  $M$ , there exists either a fixed point in  $M$  or an  $F$ -set  $F$  such that  $F \cdot T(F)$  contains a non-degenerate continuum.*

**THEOREM 3.3:** *If  $T(M) \subset M$  is a continuous transformation of a compact continuum  $M$ , there exists a compact subset  $R$  of an  $F$ -set  $F$  of  $M$  such that  $T(R) = R$ .*

**THEOREM 3.4:** *If  $T(M) \subset M$  is a continuous transformation of a compact continuum  $M$  which carries each  $F$ -set into a subset of an  $F$ -set—if, for example, the inverse of no point separates an  $F$ -set in  $M$ ,—then there exists an  $F$ -set  $F$  such that  $T(F) \subset F$ .*

In case every  $F$ -set is degenerate, we have from 3.4 the Scherrer fixed point theorem for dendrites.<sup>6</sup> If  $T$  is a homeomorphism and  $M$  is locally connected, 3.4 is Ayres' theorem.<sup>7</sup>

<sup>1</sup> Presented to the Amer. Math. Soc., Dec., 1938. The paper in full was offered to *Fundamenta Mathematicae* for publication in June, 1939.

<sup>2</sup> Compare with Kuratowski and Whyburn, *Fund. Math.*, 16, 305–331 (1930), and Moore, R. L., *Foundations of Point Set Theory*, p. 72.

<sup>3</sup>  $p \in M$  is regular if there exists an arbitrarily small neighborhood of  $p$  with a finite boundary. See Menger, *Kurventheorie*, p. 96.

<sup>4</sup> Whyburn, G. T., *Trans. Amer. Math. Soc.*, 30, 597–609 (1925).

<sup>5</sup> A set  $N \subset M$  is a retract of  $M$  if there exists a continuous transformation  $T(M) = N$  such that  $T$  is the identity transformation on  $N$ . See Borsuk, K., *Fund. Math.*, 17, 155 (1931).

<sup>6</sup> *Math. Zeit.*, 25, 129 (1926).

<sup>7</sup> *Fund. Math.*, 16, 333–336 (1930).

## MINIMAL SURFACES SPANNING CLOSED MANIFOLDS

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The great variety of phenomena presented by the Plateau-Douglas problem is surpassed by the possibilities in the corresponding problems with "free boundaries." In a previous note<sup>1</sup> a simply connected minimal surface of least area was constructed whose boundary consisted partly of a



given Jordan arc and partly of a point-set free on a given manifold,  $M$ . In a more detailed paper<sup>2</sup> a doubly connected minimal surface of least area is constructed one of whose boundaries is a prescribed Jordan curve, the other free on a given manifold. The solution of these variational problems is based on a general convergence theorem concerning boundary values of harmonic vectors. (See I or II.)

The present note is concerned with minimal surfaces no part of whose boundary is required to be a monotonically described Jordan curve. Here, as already pointed out in II, an entirely new element enters into the problem and into the existence proof: namely, it becomes necessary to specify the topological position of the required solution relative to the prescribed boundary manifold. This is done by considering linking numbers between the boundary components of the surfaces under consideration and pre-assigned cycles in the space complementary to the given manifold. Naturally, this viewpoint pertains in a general way to the theory of the Calculus of Variations in the Large for several independent variables and is by no means restricted to the problem of minimal surfaces alone.

In the present note we shall solve the following typical problem for the three-dimensional euclidean space with the position vector  $\mathbf{r}$ : given a closed surface  $M$  of genus  $p > 0$ , e.g., a torus; given, furthermore, a closed simple polygon  $H$  which has no points in common with  $M$  and which is linked with  $M$ , i.e., which is linked<sup>3</sup> with all individuals of a class of equivalent non-bounding cycles on  $M$ . We seek a simple connected minimal surface of least area whose boundary lies on  $M$ <sup>4</sup> and is linked with  $H$ , in a sense made precise immediately below. Without a topological condition such as this one the problem would become meaningless, since its solution would then be the degenerate surface  $\mathbf{r} = \text{const}$ . According to the choice of  $H$  we can, for instance, characterize surfaces filling out the hole in a torus, or spanning the inside of the torus.

Accordingly, we suppose our admissible surfaces to be represented parametrically by  $\mathbf{r}(u, v)$  or  $\mathbf{r}(r, \theta)$  in the unit circle  $B$  of the  $u, v$ -plane with polar coordinates  $r, \theta$ . Since the boundary of  $\mathbf{r}$  need not be a continuous curve on  $M$  we impose our linking condition in the following manner: we require that all images under  $\mathbf{r}(u, v)$  of simple closed curves in  $B$  sufficiently near to the circumference  $C$  of  $B$  shall be curves arbitrarily near to  $M$  and linked with  $H$ . Furthermore, we suppose that the first derivatives  $\mathbf{r}_u$  and  $\mathbf{r}_v$  are piecewise continuous in  $B$  and that the Dirichlet integral

$$\begin{aligned} D[\mathbf{r}] &= D_B[\mathbf{r}] = \frac{1}{2} \int \int_B (\mathbf{r}_u^2 + \mathbf{r}_v^2) du dv \\ &= \frac{1}{2} \int_0^{2\pi} \int_0^1 \left( \mathbf{r}_r^2 + \frac{1}{r^2} \mathbf{r}_\theta^2 \right) r dr d\theta \end{aligned}$$

exists. We then establish the variational problem: to find an admissible vector  $\mathfrak{z}$  for which

$$D[\mathfrak{z}] = d$$

is the smallest possible value. That a solution of this problem yields a minimal surface follows exactly as in the case of the Plateau problem, either with or without the use of conformal mapping. It is the existence proof which requires an essentially new reasoning.

*I. Existence Proof.*—Denoting by  $d$  the greatest lower bound of  $D[\mathfrak{z}]$  for admissible vectors we define an “admissible sequence”  $\mathfrak{z}_n$  as a sequence all of whose members satisfy the admissibility conditions except that the boundary of the surface  $\mathfrak{z}_n$  need not be on  $M$ , but only on a manifold  $M_n$  which, for  $n \rightarrow \infty$ , tends to  $M$  in the sense that the greatest distance from points of  $M_n$  to  $M$  tends to zero. Let  $\delta$  denote the greatest lower bound of  $D[\mathfrak{z}]$  for all such admissible sequences. Then an admissible sequence  $\mathfrak{z}_n$  for which

$$D[\mathfrak{z}_n] \rightarrow \delta$$

is called a *generalized minimizing sequence*. We obviously have  $\delta \leq d$ , and we shall see that  $\delta = d$ .

For the existence proof we start with such a sequence  $\mathfrak{z}_n$  and replace it by a generalized minimizing sequence of harmonic vectors as follows: We choose  $r_n$  so close to 1 that the piecewise smooth curve  $\mathfrak{z}_n(r_n, \theta)$  defined by the parameter  $\theta$  and called  $M_n$  lies within the distance  $\epsilon = 1/n$  of  $M$  and is linked with  $H$ . We then form the harmonic surface  $\mathfrak{z}_n^*(u, v)$  which is defined on the boundary  $C$  of  $B$  by  $\mathfrak{z}_n^*(1, \theta) = \mathfrak{z}_n(r_n, \theta)$ . This surface spans  $M_n$ . Since  $D[\mathfrak{z}_n^*] \leq D[\mathfrak{z}_n]$ , the  $\mathfrak{z}_n^*$  are again a generalized minimizing sequence. Now, since  $M_n$  and  $H$  are linked, there must exist at least one point  $u_0, v_0$  in  $B$  such that  $\mathfrak{z}_n^*(u_0, v_0)$  is on  $H$ . By a complex linear transformation of  $B$  into itself we can throw  $u_0, v_0$  into the origin and obtain a harmonic vector  $\mathfrak{z}_n$  with  $D[\mathfrak{z}_n] = D[\mathfrak{z}_n^*]$  and with the boundary  $M_n$ . We operate now with the new sequence  $\mathfrak{z}_n$ .  $D[\mathfrak{z}_n]$  is uniformly bounded. Hence we can, according to an elementary lemma of potential theory, choose a subsequence for which  $\mathfrak{z}_n \rightarrow \mathfrak{z}$  uniformly in each concentric circle. The harmonic vector  $\mathfrak{z}$  has the point  $\mathfrak{z}(0, 0)$  on  $H$ . According to the usual reasoning we have

$$D[\mathfrak{z}] \leq \lim D[\mathfrak{z}_n] = \delta, \quad (1)$$

and likewise, for any subdomain of  $B$ , e.g., for the circle  $B'$ :  $r \leq 1/2$ , we have

$$2\alpha = D_{B'}[\mathfrak{z}] = D_{1/2}[\mathfrak{z}] \leq \liminf D_{1/2}[\mathfrak{z}_n]. \quad (2)$$

By the fundamental convergence theorem on boundary values in I or II the boundary of  $\mathfrak{z}$  is on  $M$ . Since  $\mathfrak{z}(0, 0)$  is at a positive distance from  $M$ , it follows that  $\mathfrak{z}$  is not constant in  $B$  or  $B'$ . Hence  $\alpha > 0$ . We therefore have, for sufficiently large  $n$ ,

$$D_{1/2}[\mathfrak{z}_n] > \alpha > 0, \quad (3)$$

with  $\alpha$  fixed.

If we show that  $\mathfrak{z}$  is admissible, then  $\mathfrak{z}$  is immediately recognized as the solution, and in addition, since  $D[\mathfrak{z}] \leq \delta \leq d$  and  $D[\mathfrak{z}] \geq d$ , the relation  $\delta = d$  is established.

All that remains to be proved—and this is the crucial point in the whole reasoning—is that the boundary of  $\mathfrak{z}$  is linked with  $H$ . To this end we first mark off the points in  $B$  for which  $\mathfrak{z}(u, v)$  is on  $H$ . Since, according to the convergence theorem in I or II, the boundary of  $\mathfrak{z}$  is on  $M$ , and hence bounded away from  $H$ , there are only a finite number of such “intersections,” while the number of these points for  $\mathfrak{z}_n$  on the other hand need not be bounded. For a given small  $\epsilon$  we choose a circle  $\rho = r_\epsilon$  which encloses all the intersection points of  $\mathfrak{z}$ , and such that  $\mathfrak{z}(\rho, \theta)$  defines a curve  $M_\epsilon$  everywhere nearer to  $M$  than  $\epsilon/4$ . We then choose  $n$  so large that  $|\mathfrak{z}_n(\rho, \theta) - \mathfrak{z}(\rho, \theta)| < \epsilon/4$  and that  $\mathfrak{z}_n$  have the same number of intersections for  $r < \rho$  as  $\mathfrak{z}$ . Suppose that the curve  $\mathfrak{z}(\rho, \theta)$  is not linked with  $H$ .  $\mathfrak{z}_n(\rho, \theta)$  would then also not be linked with  $H$ . But, since the curve  $\mathfrak{z}_n(1, \theta)$  is linked with  $H$ , the algebraic sum of the intersection numbers of  $\mathfrak{z}_n$  corresponding to the ring  $R_\epsilon: \rho < r < 1$  would therefore not vanish.

Now the values  $D[\mathfrak{z}_n]$  are equally bounded by a bound  $A^2$ . Hence there exists, for each  $\mathfrak{z}_n$ , a value  $\theta = \beta$  such that

$$\frac{1}{2} \int_{1/2}^1 \left( \frac{\partial \mathfrak{z}_n}{\partial r} \right)^2 dr \leq \int_{1/2}^1 \left( \frac{\partial \mathfrak{z}_n}{\partial r} \right)^2 r dr < \frac{1}{2\pi} A^2,$$

and hence, by Schwarz's inequality,

$$|\mathfrak{z}_n(r, \beta) - \mathfrak{z}_n(1, \beta)|^2 < (1 - r) \frac{A^2}{\pi}.$$

Hence the oscillation of  $\mathfrak{z}_n(r, \beta)$  in the segment  $S: \theta = \beta$  for  $\rho \leq r \leq 1$  is less than  $\epsilon/4$  if  $\rho$  is chosen near enough to 1; consequently the values of  $\mathfrak{z}_n$  on the segment  $S$  are at a distance less than  $\epsilon/2$  from  $M$ , since  $\mathfrak{z}_n(1, \beta)$  is on  $M$ . We cut the ring  $R$  along  $S$  and thus obtain a simply connected domain  $R^* = R_\epsilon^*$  whose boundary is mapped by  $\mathfrak{z}_n$  on a continuous curve nearer to  $M$  than  $\epsilon$  and linked with  $H$ . (No intersection points of  $\mathfrak{z}_n$  can correspond to points on  $S$ , since such points are farther away from  $M$  than  $\epsilon$ .) We have

$$D_{R^*}[\xi_n] = D[\xi_n] - D_{B-R^*}[\xi_n]$$

and

$$D_{B-R^*}[\xi_n] > D_{1/2}[\xi_n] > \alpha$$

because of (3), hence

$$D_{R^*}[\xi_n] < D[\xi_n] - \alpha$$

If we let  $\epsilon$  tend to zero and, accordingly,  $n$  to infinity and  $\rho$  to 1, we have

$$\liminf D_{R^*}[\xi_n] \leq \delta - \alpha. \quad [4]$$

But, by a conformal mapping  $R^*$  can be transformed into the unit circle  $B$  and  $\xi_n$  in  $R^*$  into a vector  $\delta_n$  in  $B$  with  $D[\delta_n] = D_{R^*}[\xi_n]$ . The sequence  $\delta_n$  is certainly an admissible sequence. Hence

$$\liminf D[\delta_n] = \liminf D_{R^*}[\xi_n] \geq \delta,$$

which contradicts (4). Therefore our assumption that  $\xi$  is not admissible is refuted and the existence proof completed.

*II. Remarks.*—(1) If we consider the special case where  $M$  degenerates into a Jordan curve, we obtain by our method the solution of a problem similar to the Plateau problem, but different in so far as a much wider class of surfaces is admitted to competition. But, as stated before in II, this more general problem leads to the same solution as the Plateau problem.<sup>5</sup>

(2) The solution of the variational problem satisfies a natural boundary condition expressing orthogonality in a certain average sense.<sup>6</sup>

(3) The problem and method of this paper lead to a variety of generalizations. We can consider minimal surfaces not only having prescribed topological structure, but also having prescribed linking properties, e.g., prescribed linking numbers of boundary elements with different preassigned cycles linked with homology classes on  $M$ . The solution of problems thus specified depends on sufficient conditions in the form of inequalities similar to those known from the Plateau-Douglas problem; and, in addition, inequalities referring to the topological structure of the minimal surface relative to the manifold  $M$ . The result in all cases is as follows: A solution of a prescribed topological type with prescribed linking numbers exists if the lower bound for the areas under these conditions is strictly smaller than for other (not necessarily lower) linking numbers and for lower topological type of surfaces.<sup>7</sup>

<sup>5</sup> Courant, "The Existence of a Minimal Surface of Least Area Bounded by Prescribed Jordan Arcs and Prescribed Surfaces," these PROCEEDINGS, 24, 97 (1938)—hereafter referred to as I.

<sup>6</sup> Courant, "The Existence of Minimal Surfaces of Given Topological Structure under

Prescribed Boundary Conditions," printed in *Acta Math.* and soon to appear. This paper will be referred to as II.

<sup>2</sup> Two simple closed curves  $C_1, C_2$  in the three-dimensional space are said to be "linked" if the algebraic sum of the intersections of  $C_2$  with an orientable surface spanning  $C_1$  (or, vice versa,  $C_1$  with a surface spanning  $C_2$ ) is different from zero. (See Alexandroff-Hopf, *Topologie*, pp. 413-426.)

<sup>3</sup> This is defined as follows: If  $\mathfrak{I}(u, v)$  is represented parametrically in a domain  $D$  of the  $u, v$ -plane having the boundary  $C$ , then the boundary values of  $\mathfrak{I}$  are said to be on  $M$  if, for every sequence  $(u_n, v_n)$  which tends to  $C$ , the distance of  $\mathfrak{I}(u_n, v_n)$  from  $M$  tends to zero.

<sup>4</sup> The proof will be given elsewhere.

<sup>5</sup> See II.

<sup>7</sup> A detailed paper by N. Davids on these questions will be published later.

## ON CAUCHY'S INTEGRAL THEOREM AND ON THE LAW OF THE MEAN FOR NON-DERIVABLE FUNCTIONS

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In a recent very interesting paper, printed in these PROCEEDINGS, 25, 621 (1939), Professor Menger studies a fundamental question. In a rectangle  $R$  let  $p(x, y)$  and  $q(x, y)$  be two continuous functions; we associate with each rectifiable curve  $C$  the number  $J(C) = \int_C (pdx + qdy)$ . Under which condition is  $J$  the same for any coterminal curves in  $R$ ? (In other words, under which condition is  $pdx + qdy$  an exact differential?) It is sufficient to study here the broken lines  $C$ , the sides of which are parallel to the  $x$ -axis or to the  $y$ -axis. Therefore we will only study under which condition the preceding integral is equal to zero, when the path of integration is a rectangle, whose sides are parallel to the axes, for instance, when the path of integration is the boundary of  $R$ .

I find here a new simple condition, which is both necessary and sufficient, and add some other simple remarks.

1. With Menger's notations we suppose that  $R$  is the rectangle  $a \leq x \leq b, c \leq y \leq d$ . If we arbitrarily choose some numbers  $x_i, y_j$  such that

$$a = x_0 < x_1 < \dots < x_m < x_{m+1} = b,$$

$$c = y_0 < y_1 < \dots < y_n < y_{n+1} = d,$$

we will say that the points  $(x_i, y_j)$  define a rectangular net in  $R$ . The rectangle  $R$  will be divided into partial rectangles  $r_{ij}$ , whose vertices are the points

$$(x_i, y_j), (x_{i+1}, y_j), (x_{i+1}, y_{j+1}), (x_i, y_{j+1}), (i \leq m), (j \leq n).$$

The sides of this net are the segments joining two points

$$(x_i, y_j) \text{ and } (x_{i+1}, y_j) \text{ or two points } (x_i, y_j), (x_i, y_{j+1}).$$

By changing Menger's definition, we will say that we have *dotted* the net, if we have chosen a point on every side: a point  $(\xi_{ij}, y_j)$  on the side joining  $(x_i, y_j)$  and  $(x_{i+1}, y_j)$  and a point  $(x_i, \eta_{ij})$  on the side joining the points  $(x_i, y_j)$  and  $(x_i, y_{j+1})$ . Consequently

$$x_i \leq \xi_{ij} \leq x_{i+1}; \quad y_j \leq \eta_{ij} \leq y_{j+1}. \quad (1)$$

We will consider the ratios

$$\frac{\Delta p}{\Delta y} = \frac{p(\xi_{i,j+1}, y_{j+1}) - p(\xi_{ij}, y_j)}{y_{j+1} - y_j} \quad (2)$$

and

$$\frac{\Delta q}{\Delta x} = \frac{q(x_{i+1}, \eta_{i+1,j}) - q(x_i, \eta_{ij})}{x_{i+1} - x_i} \quad (3)$$

and their *difference*

$$\delta_{ij} = \left| \frac{\Delta p}{\Delta y} - \frac{\Delta q}{\Delta x} \right|.$$

For the sake of simplicity, we will also write

$$p(\xi_{ij}, y_j) = p_{ij}; \quad q(x_i, \eta_{ij}) = q_{ij}$$

$$\frac{\Delta p}{\Delta x} = \frac{p_{i,j+1} - p_{ij}}{y_{j+1} - y_j}, \quad \frac{\Delta q}{\Delta y} = \frac{q_{i+1,j} - q_{ij}}{x_{i+1} - x_i}. \quad (4)$$

If we consider other points  $\bar{\xi}_{ij}, \bar{\eta}_{ij}$  (where  $x_i \leq \bar{\xi}_{ij} \leq x_{i+1}$ , and  $y_j \leq \bar{\eta}_{ij} \leq y_{j+1}$ ), we will write

$$\bar{p}_{ij} = p(\bar{\xi}_{ij}, y_j); \quad \bar{q}_{ij} = q(x_i, \bar{\eta}_{ij}). \quad (4)\text{bis}$$

We can now state the condition, which is both *necessary* and *sufficient*: If  $\epsilon > 0$ ,  $\sigma > 0$  are two positive numbers, arbitrarily small, we can find a dotted net, such that *the lengths of all its sides are less than  $\epsilon$ , and all the differences  $\delta$  are less than  $\sigma$ .*

From the following proof it will appear evident that, in the preceding condition, we might disregard the number  $\sigma$  and say that *all the differences  $\delta$  are equal to zero.*

*The condition is necessary:* Let us suppose that  $p, q$  are arbitrary continuous functions. According to the law of the mean, we can choose the numbers  $\bar{\xi}_{ij}, \bar{\eta}_{ij}$  in such a way that



$$\int_{x_i, y_j}^{x_{i+1}, y_j} p(x, y) dx = (x_{i+1} - x_i) p(\bar{\xi}_{ij}, y_j) = (x_{i+1} - x_i) \bar{p}_{ij}$$

$$\int_{x_i, y_j}^{x_i, y_{j+1}} q(x, y) dy = (y_{j+1} - y_j) q(x_i, \bar{\eta}_{ij}) = (y_{j+1} - y_j) \bar{q}_{ij}$$

$$(x_i < \bar{\xi}_{ij} < x_{i+1}), \quad (y_j < \bar{\eta}_{ij} < y_{j+1}).$$

We indicate now by  $\int_R$  or  $\int_r$  the values of the integral of  $pdx + qdy$ , when the path of integration is the positive boundary of  $R$  or of  $r = r_{ij}$ . And we find immediately that

$$\int_r = -(x_{i+1} - x_i)(\bar{p}_{i, j+1} - \bar{p}_{ij}) + (y_{j+1} - y_j)(\bar{q}_{i+1, j} - \bar{q}_{ij}). \quad (5)$$

If  $pdx + qdy$  is an exact differential, this integral is equal to zero; and therefore (5) proves that  $\delta = 0$ , if we choose the dotting points  $\xi, \eta$ , by supposing that  $\xi_{ij} = \bar{\xi}_{ij}, \eta_{ij} = \bar{\eta}_{ij}, (p = \bar{p}, q = \bar{q})$ . (Obviously  $\delta < \sigma$ , since  $\delta = 0$ .) Before we prove that the condition is also sufficient, it may be useful perhaps to study the differential meaning of the ratios (2) and (3). Let us, for instance, study the former, and let  $x_i$  be a constant, while  $y$  and  $x_{i+1} - x_i = h$  are variable. The point  $\xi$  such that

$$\int_{x_i}^{x_{i+1}} p(x, y) dx = hp(\xi_{ij}, y) = h\bar{p}_{ij} \quad (6)$$

$$(x_i \leq \bar{\xi}_{ij} < x_{i+1} = x_i + h)$$

is a function  $\bar{\xi}(y, h)$  of  $y$  and  $h$ . We can completely determine it, even if there are many points  $\xi$  satisfying (6); it is sufficient to choose the least; which is possible, because  $p$  is continuous. If we suppose that  $x = \bar{\xi}$ , the function  $p(x, y)$  becomes a function  $P(h, y)$  of  $h, y$ . When  $y_{j+1}$  approaches  $y_j$ , the ratio (3) approaches the limit

$$\frac{q(x+h, y) - q(x, y)}{h}; \quad (x = x_i, y = y_j) \quad (x+h = x_{i+1}) \quad (7)$$

and consequently also the ratio (2), which is equal to (3), approaches the same limit, which is therefore equal to  $\frac{\partial P}{\partial y}$ . (Here  $\xi = \bar{\xi}, \eta = \bar{\eta}$ .)

From what we have already proved we deduce consequently: It may happen that  $p(x, y)$  is a non-derivable function of  $y$ , when we suppose that  $x = \text{const.}$  But *the derivative of  $p$  with respect to  $y$  exists if* [instead of supposing that  $x = \text{const.}$ ] we suppose that  $x = \bar{\xi}(y, h)$ . And this derivative is equal to (7).

The condition is sufficient. Let us suppose that  $\delta < \sigma$  (for every one of the rectangles  $r = r_{ij}$ ). From (5) we infer that

$$\int_r = A_{ij} - (x_{i+1} - x_i) \{ [\bar{p}_{i,j+1} - \bar{p}_{ij}] - [p_{i,j+1} - p_{ij}] \} + \\ + (y_{j+1} - y_j) \{ [\bar{q}_{i,j+1} - \bar{q}_{ij}] - [q_{i,j+1} - q_{ij}] \}, \quad (8)$$

where

$$A_{ij} = -(x_{i+1} - x_i)(p_{i,j+1} - p_{ij}) + (y_{j+1} - y_j)(q_{i+1,j} - q_{ij})$$

is deduced from the second member of (5), by writing  $\xi, \eta$  instead of  $\bar{\xi}, \bar{\eta}$ . Since  $\delta < \sigma$ , we get

$$|A| < \sigma(x_{i+1} - x_i)(y_{j+1} - y_j). \quad (9)$$

We remark, moreover, that, for instance:

$$\sum_j (\bar{p}_{ij} - \bar{p}_{i,j+1}) = \bar{p}_{i0} - \bar{p}_{i,n+1} = p(\bar{\xi}_{i0}, c) - p(\bar{\xi}_{i,n+1}, d). \quad (10)$$

For the sake of brevity, we do not write the analogous equations for  $p_{ij}, \bar{q}_{ij}, q_{ij}$ . From (9) and (10) we can now immediately deduce that

$$\int_R = \sum \int_r = \sum A_{ij} + \sum_i (x_{i+1} - x_i) [(\bar{p}_{i0} - \bar{p}_{i,n+1}) - \gamma - \\ - (p_{i0} - p_{i,n+1})] + \sum_j (y_{j+1} - y_j) [(\bar{q}_{m+1,} - \bar{q}_{0j}) - (q_{m+1,} - q_{0j})]. \quad (11)$$

From (9) we deduce that

$$|\sum A_{ij}| < \sigma(b - a)(d - c).$$

Since  $p(x, c)$  is continuous and therefore integrable, the sum

$$\sum (x_{i+1} - x_i) [\bar{p}_{i0} - p_{i0}] = \sum (x_{i+1} - x_i) [p(\bar{\xi}_{i0}, c) - p(\xi_{i0}, c)] \\ (x_i \leq \xi_{i0} \leq x_{i+1}) \quad (x_i \leq \bar{\xi}_{i0} < x_{i+1})$$

becomes infinitesimal, when the greatest side of our net approaches zero. In the same way we can study the other terms of the second member of (11).

And we prove by this method that  $\int_R$  is arbitrarily small, by choosing  $\epsilon$

and  $\sigma$  sufficiently small. Therefore  $\int_R = 0$ ; which we had to demonstrate.

2. *The Law of the Mean.*—Let us write

$$\int_r = - \int_{x_i}^{x_{i+1}} [p(y_{j+1}, x) - p(y_j, x)] dx + \int_{y_j}^{y_{j+1}} [q(x_{i+1}, y) - q(x_i, y)] dy$$

$$x = x_i, y_j = y, x_{i+1} - x_i = h, y_{j+1} - y_j = k.$$

From the equation  $\int_r = 0$  we deduce, by using the usual law of the mean for the integrals of the continuous functions

$$p(y_{j+1}, x) - p(y_j, x) \text{ and } q(x_{i+1}, y) - q(x_i, y)$$

that, for every rectangle  $r$ , we can find two numbers  $\theta, \theta'$  satisfying the inequalities  $0 < \theta < 1, 0 < \theta' < 1$  such that

$$\frac{p(y + k, x + \theta h) - p(y, x + \theta h)}{k} = \frac{q(x + h, y + \theta' k) - q(x, y + \theta' k)}{h}.$$

The question as to whether this condition is also sufficient is still unanswered.

This condition is equivalent to the law of the mean; by remarking that  $\varphi(x + y)(dx + dy)$  is an exact differential, if  $\varphi$  is a continuous function, even if it is not derivable, we deduce a *law of the mean* for the continuous functions  $\varphi(z)$ , even if they are not derivable. The proof is quite elementary and will be printed in another periodical. I will be contented to state here the final result without demonstration.

If  $\varphi(z)$  is continuous in the closed interval  $a \leq z \leq a + H$ , we get:

$$\frac{\varphi(a + H) - \varphi(a)}{H} = \frac{\varphi(a + \theta H + h) - \varphi(a + \theta H)}{h}$$

where  $h$  is an arbitrary sufficiently small number, and (if  $\varphi, a, H$  are given)  $\theta$  is a function of  $h$  with following properties:

$\alpha)$   $0 < \theta < 1$ .

$\beta)$  The point  $a + \theta h$  approaches a limit, if  $h$  becomes infinitesimal.

This law becomes equivalent to the usual law of the mean, if  $\varphi(x)$  is a derivable function.

3. *Fourier's Coefficients.*—But we can also consider our question from another point of view. If  $p, q$  are continuous functions, and  $pdx + qdy$  is an exact differential, for instance the function  $p$  is not an arbitrary continuous function. This is obvious, because in this case

$$\int_a^b p(x, y) dx \quad (a, b = \text{const.})$$

must be a function, which possesses a derivative  $q(b, y) - q(a, y)$  with respect to  $y$ . And when  $\varphi(x)$  is a function of  $x$ , with a continuous derivative  $\varphi'(x)$ , also

$$\int_a^b p(x, y) \varphi(x) dx = \left[ \varphi(x) \int_a^x p(x, y) dx \right]_{x=a}^{x=b} - \int_a^b \left[ \int_a^x p(x, y) dx \right] \varphi'(x) dx$$

is a function of  $y$  with a bounded derivative

$$\begin{aligned} \varphi(b)[q(b, y) - q(a, y)] - \int_a^b [q(x, y) - q(a, y)] \varphi'(x) dx = \\ \varphi(b)q(b, y) - \varphi(a)q(a, y) - \int_a^b \varphi'(x)q(x, y) dx. \end{aligned}$$

We deduce, for instance, that, if we consider  $p$  as a function of the only variable  $x$ , by supposing  $y = \text{const.}$ , and develop  $p$  in a Fourier's series, *its Fourier's coefficient must be derivable functions of  $y$* . The class of functions, which have this property, may perhaps be interesting, but I did not succeed in proving that we can choose  $p(x, y)$  arbitrarily in this class of functions.

Let us suppose, for the sake of simplicity, that the length of every side of  $R$  is  $2\pi$ , and that inside  $R$

$$a_0(y) + \sum [a_n(y) \cos nx + b_n(y) \sin nx]$$

is Fourier's development of  $p(x, y)$ . According to the preceding remark, the coefficients  $a, b$  are derivable functions of  $y$ . I *believe*, but I could not demonstrate, that (if  $pdx + qdy$  is an exact differential)  $q(x, y)$  may be defined by the development

$$\phi(y) + xa'_0(y) + \sum \left[ a'_n(y) \frac{\sin nx}{n} + b'_n(y) \frac{1 - \cos nx}{n} \right]$$

where  $\phi(y)$  is an arbitrary integrable function of  $y$ . If  $x, y$  were polar coordinates, and  $y$  were the radius vector, the formulas become simpler, because necessarily  $a_0$  is equal to zero (at least if  $pdx + qdy$  is the differential of an one-valued function and the origin belongs to the considered region).

# RECALCULATION AND EXTENSION OF THE MODULUS AND OF THE LOGARITHMS OF 2, 3, 5, 7 AND 17

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A year or more ago while the author was engaged in extending the work of J. C. Adams<sup>1</sup> by computing the Napierian logarithms of 11, 13, 17, 19, 23, 29 and 31 to a fairly large number of decimal places, but before the calculations had been completed, he became convinced that it would not be justifiable to claim for the new values complete absence of error unless the basic data, namely, the logarithms of 2, 3, 5 and 7, were proved to be correct as printed. This conclusion was reached after a careful search through the available literature failed to show that in the interim any other mathematician had repeated or independently verified the values found by Adams. In order to contribute more than the mere checking of the classical data the goal of nearly 330 decimal places was set for the present investigation. Since Adams' approximations were claimed by him to be valid to about 273 decimal places the gain proposed would be 55 figures.

The notation used here is the same as that employed by me in an earlier paper.<sup>2</sup> The actual work involved the following steps in the order stated: (a) the selection of suitable pairs of numbers  $(p, q)$  which satisfy the condition  $p - q = 1$ ; (b) the calculation of terms of the simple geometrical series  $\Sigma(2p - 1)^{-(2m + 1)}$ ,  $m = 0, 1, 2, 3, \dots$ ; (c) copying from the Monroe computing machine (in black ink) the numerical values of these terms on alternate horizontal lines of rectangularly ruled paper; (d) forming the sum of these data and comparing it with the previously computed value of  $S_\infty$ , that is, the limit  $(2p - 1)/4p(p - 1)$ ; (e) dividing each value of  $(2p - 1)^{-(2m + 1)}$  by  $2m + 1$  and entering the quotients (in red ink) in the blank lines directly below the corresponding terms of the geometrical series; (f) summing separately the lines which represent the even and odd terms of the final series,  $s(+) = \Sigma_0 (2p - 1)^{-(4m + 1)}/(4m + 1)$  and  $s(-) = \Sigma_1 (2p - 1)^{-(4m - 1)}/(4m - 1)$ ; and (g) evaluation of  $\log(p/q)$  and  $\tan^{-1}[1/(2p - 1)]$ , respectively, as  $2[s(+) + s(-)]$  and  $s(+) - s(-)$ . All quotients were checked by multiplication, the results of addition were tested by repetition with the successive groups of ten figures staggered five decimal places, and throughout all stages of the work every precaution known to me was taken to eliminate errors.

The pairs of numbers chosen for the calculation of the logarithms  $l_2, l_3, l_5, l_7$  and  $l_{17}$  are given in table 1.

TABLE 1

$2p - 1$	$p$	$q$
5	3	2
239	$120 = 2^3 \cdot 3 \cdot 5$	$119 = 7 \cdot 17$
577	$289 = 17^2$	$288 = 2^5 \cdot 3^2$
2449	$1225 = 5^2 \cdot 7^2$	$1224 = 2^3 \cdot 3^2 \cdot 17$
4999	$2500 = 2^2 \cdot 5^4$	$2499 = 3 \cdot 7^2 \cdot 17$
8749	$4375 = 5^4 \cdot 7$	$4374 = 2 \cdot 3^7$

The selection of these particular numbers was based not only upon the essential condition that they should have the prime factors 2, 3, 5 and 7 but also upon other considerations. For example, 5 and 239 lead to  $\tan^{-1}(1/5)$  and  $\tan^{-1}(1/239)$ , that is, to numbers which can be checked either directly and separately by comparison with the values calculated by Rutherford<sup>3</sup> and Shanks<sup>4</sup> or indirectly and collectively with the standard value of  $\pi$  through Machin's formula. The choice of  $2p - 1 = 239$  or  $q = 7 \cdot 17$  obviously introduced the number 17 into the work. Since  $(2449)^2 = 5997601 = 6 \times 10^6 - 2 \times 10^3 - 4 \times 10^2 + 1 \times 10^0$  the quotients constituting the terms of the geometrical series of which  $1/(2449)^2$  is the common ratio can be checked rapidly by multiplication. In fact the number of unit strokes of the computing machine in this case is only 13 ( $= 6 + 2 + 4 + 1$ ). Similarly  $(4999)^2 = 24990001 = 2 \times 10^7 + 5 \times 10^6 - 1 \times 10^4 + 1 \times 10^0$  which requires only 9 strokes for every ten consecutive digits in the quotient.

For brevity let  $a_1 = S(1/5)$ ,  $a_2 = S(1/239)$ ,  $a_3 = S(1/2449)$ ,  $a_4 = S(1/4999)$  and  $a_5 = S(1/8749)$ . Then the required logarithms are given by the following set of independent linear equations:

$$\left. \begin{aligned} -1l_2 + 1l_3 &= 2a_1 \\ 3l_2 + 1l_3 + 1l_5 - 1l_7 - 1l_{17} &= 2a_2 \\ -3l_2 - 2l_3 + 2l_5 + 2l_7 - 1l_{17} &= 2a_3 \\ 2l_2 - 1l_3 + 4l_5 - 2l_7 - 1l_{17} &= 2a_4 \\ -1l_2 - 7l_3 + 4l_5 + 1l_7 &= 2a_5 \end{aligned} \right\} \tag{1}$$

These equations are satisfied identically by the expressions:

$$\left. \begin{aligned} l_2 &= (27a_1 + 18a_2 - 7a_3 - 11a_4 + 10a_5)/8 \\ l_3 &= (43a_1 + 18a_2 - 7a_3 - 11a_4 + 10a_5)/8 \\ l_5 &= (63a_1 + 26a_2 - 11a_3 - 15a_4 + 18a_5)/8 \\ l_7 &= (76a_1 + 40a_2 - 12a_3 - 28a_4 + 24a_5)/8 \\ l_{17} &= (111a_1 + 42a_2 - 27a_3 - 31a_4 + 34a_5)/8 \end{aligned} \right\} \tag{2}$$

In each of the preceding parentheses the coefficients of  $a_1$  and  $a_2$  are larger than those of  $a_3$ ,  $a_4$  and  $a_5$  wherefore  $a_1$  and  $a_2$  were calculated to 336 and 335 decimal places, respectively, while the three remaining  $a$ 's were rounded off at the 330th place.



After the  $l$ 's had been computed from formulas (2) the values obtained were found by substitution to satisfy equations (1) as far as and beyond the 333d decimal place. This agreement merely means that no mistake was made while substituting the numbers in formulas (2) and it would have been obtained even if one or more of the  $a$ 's and the dependent  $l$ 's were vitiated by errors. Hence the necessity for discovering if possible an identity which could only be fulfilled by a set of perfect  $a$ 's became imperative.

An exhaustive inspection of the British Association Factor Table<sup>6</sup> from 1 to 50,000 (that is, throughout the range of numbers whose squares did not exceed the capacity of the computing machines employed) showed that (289, 288) is the largest pair of consecutive integers which fulfil the following conditions: (i) one member of the pair shall be a multiple of 17; (ii) all the remaining prime factors of the pair shall be comprised in the group 1, 2, 3, 5 and 7; (iii) the pair must lead to an equation which, when combined with equations (1) will give an identity connecting *all* of  $a_1, a_2, a_3, a_4$  and  $a_5$ . Since  $289 = 17^2$  and  $288 = 2^5 \cdot 3^2$  the equation in question is

$$-5l_2 - 2l_3 + 2l_{17} = 2a_6 \quad (3)$$

where  $a_6 = S(1/577)$ , and the analytical condition for the compatibility of the six equations is the required identity, namely

$$a_1 - 42a_2 - 5a_3 + 15a_4 - 2a_5 - 16a_6 = 0. \quad (4)$$

This formula can also be obtained by equating the logarithms of both sides of the following identity the truth of which can be tested at once by replacing the composite numbers by their prime factors as given in table 1.

$$\left(\frac{3}{2}\right)^{-1} \left(\frac{120}{119}\right)^{42} \left(\frac{1225}{1224}\right)^5 \left(\frac{2500}{2499}\right)^{-15} \left(\frac{4375}{4374}\right)^2 \left(\frac{289}{288}\right)^{16} = 1. \quad (5)$$

Incidentally the smaller pair of numbers ( $p = 256 = 2^8, q = 255 = 3 \cdot 5 \cdot 17$ ) leads to the alternative identity

$$a_1 - 58a_2 + 11a_3 + 31a_4 - 18a_5 + 16a_7 = 0 \quad (6)$$

in which  $a_7 = S(1/511)$  converges even more slowly than  $S(1/577)$ . With reference to rate of convergence the fact may merit recording that in order to increase the number of decimal places for  $S(1/577)$  from 273 to 328—a gain of about 20 per cent—it was necessary to augment by over 51 per cent the number of figures actually written in the terms of the series.

Attention will now be turned to the quantitative evidence for the degree of accuracy both of the final data published by J. C. Adams and of the numbers involved at various stages in the present work. The "errors" in Adams' constants are entirely terminal and they are given in table 2.

TABLE 2

CONSTANT	ERROR
log 2	$+4.43 \times 10^{-275}$
log 3	$+6.95 \times 10^{-275}$
log 5	$+1.01 \times 10^{-274}$
log 7	$+1.44 \times 10^{-274}$
log 10	$+1.45 \times 10^{-274}$
$M$	$-9.46 \times 10^{-273}$

It is interesting to note that all of his logarithms have errors of the same sign. With characteristic perspicacity Adams<sup>6</sup> wrote "And finally the corrected value of the Modulus is  $M = 0.43429 \dots 21868\ 25$  which is true, certainly to 272 and probably to 273 places of decimals."

The geometrical series test yielded the first six of the equations transcribed below. It was not applied to the functions of  $1/5 (= 2 \times 10^{-1})$  since their evaluation depended explicitly upon positive integral powers of 2. These involutions had been thoroughly verified by the writer in the year 1900.

$$\begin{aligned}
 +S_{\infty}(1/239) - \sum_0^{34} (239)^{-(4m+1)} &= 1.6 \times 10^{-335} & S_{\infty}(1/2449) - \sum_0^{48} (2449)^{-(2m+1)} &= 3.6 \times 10^{-330} \\
 -S_{\infty}(1/239) - \sum_1^{35} (239)^{-(4m-1)} &= 1.2 \times 10^{-335} & S_{\infty}(1/4999) - \sum_0^{44} (4999)^{-(2m+1)} &= 1.3 \times 10^{-330} \\
 S_{\infty}(1/577) - \sum_0^{60} (577)^{-(2m+1)} &= 2.0 \times 10^{-335} & S_{\infty}(1/8749) - \sum_0^{41} (8749)^{-(2m+1)} &= 3.0 \times 10^{-330} \\
 [\tan^{-1}(1/5)]_S - [\tan^{-1}(1/5)]_U &= 4.0 \times 10^{-336} \\
 [\tan^{-1}(1/239)]_S - [\tan^{-1}(1/239)]_U &= -1.6 \times 10^{-335} \\
 \pi_S - \pi_U &= 1.3 \times 10^{-334}
 \end{aligned}$$

The subscripts  $S$  and  $U$  refer to Shanks and Uhler, respectively.

Substitution of the newly extended values of  $l_2$ ,  $l_3$  and  $l_{17}$  in equation (3), a relation which was not used in the calculation of these logarithms, led to the following significant result:

$$2l_{17} - 5l_2 - 2l_3 - 2a_6 = 4.05 \times 10^{-331}.$$

The left-hand member of the important identity (4) was evaluated to be  $3.2 \times 10^{-330}$ . This admissible error is ascribable solely to the trinomial  $15a_4 - 5a_3 - 2a_6$  since each of  $a_1$ ,  $a_2$  and  $a_6$  was carried as far as the 335th decimal place while  $a_3$ ,  $a_4$  and  $a_5$  were rounded off at the 330th place. Fi-

nally in checking the value of the modulus by forming the product  $M \cdot l_{10}$  a continuous succession of 336 nines was obtained.

The newly extended values of the natural logarithms of 2, 3, 5, 7 and 17, and of the common modulus are collected in table 3. These approximations are certainly correct to 328 decimal places and their unreliability probably does not exceed one or two units in the 329th place. If at any future time the values of the discrete sums of the positive and negative terms of the infinite series involved in the present work should be desired they may be obtained directly by first calculating the  $a$ 's through substitution of the tabulated  $l$ 's in equations (1) and then employing the following formulas:

$$\begin{aligned} s_j(+) &= (a_j + \tan^{-1} \theta_j)/2 \\ s_j(-) &= (a_j - \tan^{-1} \theta_j)/2 \end{aligned} \tag{7}$$

In addition to the arc tangents of the reciprocals of the integers 577, 2449, 4999 and 8749 table 3 gives the values of  $\tan^{-1}(1/451)$  and  $\tan^{-1}(1/10081)$ , and also the extensions of certain other constants which were published by me in an earlier paper.<sup>7</sup> These two arc tangents have just been computed from the series underlying, respectively, the values of  $\log 113$  and  $\log 71$  which were used in calculating  $\log \pi$  from Ramanujan's expression.<sup>8</sup> The extensions were finished on May 18, 1937, while retesting a table of reciprocals of factorials. They are printed in table 3 in such a manner as to fit and continue the corresponding numbers as printed on pages 433 and 434 of the monograph<sup>7</sup> in question. The accuracy of the extended numbers is measured by the right-hand members of the following test equations:

$$\begin{aligned} 1 - (e^{-10}) \times (e^{+10}) &= 5.0 \times 10^{-295}, \\ \cos^2 10 + \sin^2 10 - 1 &= 4.3 \times 10^{-288}. \end{aligned}$$

TABLE 3  
log<sub>e</sub> 2 =

0.69314	71805	59945	30941	72321	21458	17656	80755	00134	38025
52541	20680	00949	33936	21969	69471	56058	63326	99641	86875
42001	48102	05706	85733	68552	02357	58130	55703	26707	51635
07596	19307	27570	82837	14351	90307	03862	38916	73471	12335
01153	64497	95523	91204	75172	68157	49320	65155	52473	41395
25882	95045	30070	95326	36664	26541	04239	15781	49520	43740
43038	55008	01944	17064	16715	18644				

TABLE 3 (Continued)

 $\log_3 =$ 

1.09861	22886	68109	69139	52452	36922	52570	46474	90557	82274
94517	34694	33363	74942	93218	60896	68736	15754	81373	20887
87970	02906	59578	65742	36800	42259	30519	82105	28018	70767
27741	06031	62769	18338	13671	79373	69884	43609	59903	74257
03167	95911	52114	55919	17750	67134	70549	40166	77558	02222
03170	25294	68975	60690	10652	15056	42868	13803	63173	73298
57778	23669	91654	79213	18181	49019	5			

 $\log_5 =$ 

1.60943	79124	34100	37460	07593	33226	18763	95256	01354	26851
77219	12647	89147	41789	87707	65776	46301	33878	09317	96107
99966	30302	17155	62899	72400	52293	24676	19963	36166	17463
70572	75521	79637	49718	32456	53492	85620	23415	25057	27015
51936	00879	77738	97256	88193	54071	27661	54731	22180	95279
48521	29282	13580	59722	56767	22852	87240	46158	94481	78364
67132	86739	98424	63775	95931	89422				

 $\log_7 =$ 

1.94591	01490	55313	30510	53527	43443	17972	96370	84729	58186
11884	59390	14993	75798	62752	06926	77876	58498	58787	15269
93061	69420	58511	40911	72375	22576	77786	84314	89580	95163
90077	59078	24468	10427	47833	82259	34900	84673	74412	50497
37048	53551	76783	55774	86240	15102	77418	08868	67107	51412
13480	93879	74183	10810	25182	31684	93014	07330	63932	87711
93411	21406	87692	40026	05769	35850				

 $\log_{10} =$ 

2.30258	50929	94045	68401	79914	54684	36420	76011	01488	62877
29760	33327	90096	75726	09677	35248	02359	97205	08959	82983
41967	78404	22862	48633	40952	54650	82806	75666	62873	69098
78168	94829	07208	32555	46808	43799	89482	62331	98528	39350
53089	65377	73262	88461	63366	22228	76982	19886	74654	36674
74404	24327	43651	55048	93431	49393	91479	61940	44002	22105
10171	41748	00368	80840	12647	08066				

 $\log_{17} =$ 

2.83321	33440	56216	08024	95346	17873	12653	55882	03012	58574
47872	97237	73788	22925	75800	93128	09120	94868	03750	29475
18348	26204	71870	57291	39759	28419	46738	36429	97545	65742
02127	12599	13208	07209	04790	76471	68172	51666	60296	60850
69091	96813	96134	51492	95164	19209	44718	69393	25481	33184
68944	45037	58003	15646	02993	05896	37270	00327	36297	59273
99414	82424	46984	21556	64224	34393	5			

TABLE 3 (Continued)

$M =$

0.43429	44819	03251	82765	11289	18916	60508	22943	97005	80866
65661	14453	78316	58646	49208	87077	47292	24949	33843	17483
18706	10674	47663	03733	64167	92871	58963	90656	92210	64662
81226	58521	27086	56867	03295	93370	86965	88266	88331	16360
77384	90514	28443	48666	76864	65860	85135	56148	21234	87653
43543	43573	17253	83562	22813	95603	04864	66523	66095	53937
73561	76323	43191	67109	91411	59790				

$\tan^{-1}(1/451) =$

0.00221	72912	66532	02363	15882	56080	23026	31380	14502	60599
17643	99214	52019	13338	55404	43675	39034	82558	58543	44395
87108	04995	88910	09632	24025	89247	92233	60230	24247	94571
84451	16722	83161	33503	39386	54494	07932	77708	39014	76315
64780	05094	35952							

$\tan^{-1}(1/577) =$

0.00173	31005	17828	99690	63239	26177	82620	68511	14980	78800
59327	89382	35699	57691	52385	74935	80734	22847	93358	70598
96356	61775	68614	26967	56613	21839	35078	77394	53724	71418
35572	52796	24166	64413	86399	69167	26081	27592	39196	59869
64098	11494	99282	54992	61438	31419	33135	35109	63451	57505
18848	56578	01725	67976	90962	00362	69313	12814	02928	71699
62913	81132	13787	97716	84343	90302	82074			

$\tan^{-1}(1/2449) =$

0.00040	83299	07889	84408	18481	31992	99044	00572	06737	74283
70053	38677	45144	46042	13461	35015	77405	60982	70932	45713
04305	99949	52406	66320	25439	93760	94545	22519	84677	64435
46704	46028	26736	37875	65360	61246	63820	16585	22183	67056
63190	96352	77188	35207	98027	61056	13926	78570	20865	44049
90325	26335	10182	52182	64165	03332	27936	85681	33796	89799
93579	54457	16861	92622	49627	60087				

$\tan^{-1}(1/4999) =$

0.00020	00400	05333	83307	72479	85371	42948	19785	33228	97143
44926	08256	32095	52603	39634	32975	44539	51845	47049	06238
12923	63094	42014	32150	25583	78435	98399	16704	85072	75018
36338	02163	03169	96623	07518	40270	67119	84057	80148	26486
18610	47735	14108	65609	41020	87318	86948	59472	63931	90140
76445	02147	90530	51061	43414	50219	10796	83471	52085	58089
23900	19074	14132	95931	36220	37343				

TABLE 3 (Continued)

tan <sup>-1</sup> (1/8749) =									
0.00011	42987	76505	34497	95406	31656	54433	41572	51039	85186
41170	24388	58112	13047	19377	06999	98474	56827	20239	77036
33365	59321	49574	74236	74070	56118	40358	72195	87487	08290
49969	68785	15908	88360	69720	37795	09205	49310	96750	43861
88677	13495	77313	59992	87031	98770	47933	54594	09620	68560
46114	23999	91470	93199	28975	69236	59373	30949	05572	24245
82449	35001	00294	79698	27092	87283				
tan <sup>-1</sup> (1/10081) =									
0.00009	91965	07957	54564	03985	58797	77424	49951	60255	89946
71090	71455	59679	31811	67097	06143	17935	78474	20733	28137
34792	26162	76152	55449	53213	52046	37462	68193	60929	54513
84052	28071	43737	07353	98497	65534	94325	10747	50921	23363
62373	56664	62704	6						
e <sup>+10</sup> =									
.....	...07	54381	79319	60834	04440	49342	3668(2)		
e <sup>-10</sup> =									
....	...30	56049	41570	10772	99753	54408	07940	399(4)	
sin 10 =									
.....	.....	..290	61127	67064	51048	48711	04571	26379	41468
21392	89420	87572	08458	35061	96715	0157(	96)		
cos 10 =									
.....	.....	..471	69711	71010	52082	69213	07324	18341	25670
72265	61830	11009	31356	14920	90281	4223(	32)		
cos 20 =									
.....	.....	..197	39399	70438	94194	84010	91837	43935	51103
56225	20596	81042	71693	29701	02133	9639(	40)		

<sup>1</sup> J. C. Adams, *Proc. Roy. Soc. London*, **42**, 22-25 (1887).  
<sup>2</sup> H. S. Uhler, *Proc. Nat. Acad. Sci.*, **24**, 23-30 (1938).  
<sup>3</sup> Wm. Rutherford, *Proc. Roy. Soc. London*, **6**, 274, 275 (1853).  
<sup>4</sup> Wm. Shanks, *Proc. Roy. Soc. London*, **21**, 319 (1873).  
<sup>5</sup> *Brit. Assoc. Adv. Sci., Math. Tables*, **5** (1935).  
<sup>6</sup> *Scientific Papers of John Couch Adams*, **1**, 470 (1896).  
<sup>7</sup> H. S. Uhler, *Trans. Conn. Acad. Arts Sci.*, **32**, 381-434 (1937).



# THE GREEN'S FUNCTION FOR A DIFFERENTIAL SYSTEM OF INFINITE ORDER

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In the study of the Stieltjes transform<sup>1</sup>

$$f(x) = \int_0^{\infty} \frac{\varphi(t)}{x+t} dt \quad (1)$$

the author was led to consider a certain linear differential operator of infinite order

$$L[f(x)] = \lim_{k \rightarrow \infty} \frac{(-1)^{k-1}}{k!(k-2)!} [x^{2k-1} f^{(k-1)}(x)]^{(k)}. \quad (2)$$

It was found that when this operator is applied to the function  $f(x)$ , defined by (1), it yields  $\varphi(x)$  and thus inverts the integral (1). It is easily seen that (2) is formally equivalent to<sup>2</sup>

$$L[f(x)] = xD \prod'_{n=-\infty}^{\infty} \left(1 - \frac{x D}{n}\right) f(x), \quad (3)$$

where  $D$  indicates differentiation with respect to  $x$  and the prime indicates that the factor corresponding to  $n = 0$  is omitted.

Consider now the following differential system

$$\begin{aligned} L[f(x)] &= \varphi(x) \\ \lim_{x \rightarrow 0+} x f(x) &= 0 \\ \lim_{x \rightarrow \infty} f(x) &= 0 \end{aligned} \quad (A)$$

and the corresponding homogeneous system ( $B$ ) in which  $\varphi(x)$  is replaced by zero. A fundamental system of solutions of the equation

$$L[f(x)] = 0$$

is

$$f(x) = x^n \quad (n = 0, \pm 1, \pm 2, \dots).$$

No linear combination of these solutions satisfies the boundary conditions of ( $B$ ). Hence ( $B$ ) is incompatible. That is, ( $A$ ) has a unique solution. Following the analogy with systems of finite order we should be able to find the solution by use of a Green's function.

Consider now the "truncated" systems  $(A')$  and  $(B')$  which are the same as  $(A)$  and  $(B)$ , respectively, except that  $L[f(x)]$  has been replaced by

$$L_k[f(x)] = -xD \prod_{n=-k}^{k-2} \left(1 - \frac{xD}{n}\right) f(x).$$

We recall that the Green's function<sup>3</sup>  $G_k(x, t)$  for the system  $(A')$  is for each positive  $t$  a function of  $x$  which satisfies  $(B')$  for every positive  $x$  except  $x = t$ , which is continuous with its first  $(2k - 3)$  derivatives for  $(0 < x < \infty)$ , and whose  $(2k - 2)$ th derivative is continuous there except at  $x = t$ , where it has a finite jump defined by the equation

$$\lim_{x \rightarrow t+} \frac{\partial^{2k-2}}{\partial x^{2k-2}} G_k(x, t) - \lim_{x \rightarrow t-} \frac{\partial^{2k-2}}{\partial x^{2k-2}} G_k(x, t) = (-1)^{k-1} k! (k-2)! t^{-2k+1}.$$

It may be shown that these properties serve to characterize  $G_k(x, t)$  and that the solution  $f_k(x)$  of  $(A')$  is

$$f_k(x) = \int_0^\infty G_k(x, t) \varphi(t) dt.$$

It is natural to define the Green's function  $G(x, t)$  of the system  $(A)$  as the limit as  $k$  becomes infinite of  $G_k(x, t)$  and to expect that the solution of  $(A)$  will be

$$f(x) = \int_0^\infty G(x, t) \varphi(t) dt. \quad (4)$$

We show that this conjecture is correct.

We can in fact obtain an explicit formula for  $G_k(x, t)$ . It is found to be

$$\begin{aligned} G_k(x, t) &= k \int_0^\infty \frac{(y + x - t)^{k-1} y^{k-2}}{(y + x)^{2k-1}} dy & (t < x) \\ &= k \int_0^\infty \frac{(y + t - x)^{k-2} y^{k-1}}{(y + t)^{2k-1}} dy & (t > x). \end{aligned}$$

The details of computation will be given in a later paper. Now by the Laplace method for the asymptotic evaluation of an integral, or otherwise, it may be shown that

$$\lim_{k \rightarrow \infty} G_k(x, t) = (x + t)^{-1},$$

so that the solution of  $(A)$  in the form (4) is precisely (1). That (1) actually satisfies the system  $(A)$  was proved in an earlier paper, as we observed at the beginning of this note. Thus the Stieltjes kernel may be regarded as the Green's function for a certain differential system of infinite order.

The importance of the present procedure is that it provides a straightforward method of finding the kernel that corresponds to a given differential operator. As other examples we note that if (A) is altered by omitting<sup>4</sup> the factor  $(1 + xD)$  in (3) the kernel becomes  $t(x + t)^{-2}$ ; if in addition the factor  $1 + 2^{-1}xD$  is omitted the kernel becomes  $t^2(x + t)^{-3}$ , etc. If the system (A) is altered by replacing  $L[f(x)]$  by its iterate  $L^2[f(x)]$  we find that the Green's function for the corresponding truncated system is

$$\int_0^\infty G_k(x, y)G_k(y, t)dy$$

and that the Green's function for the system of infinite order is  $[\log(x/t)] [x - t]^{-1}$ , as one would expect from earlier consideration of R. P. Boas<sup>5</sup> and the author.

<sup>1</sup> D. V. Widder, "The Stieltjes Transform," *Trans. Am. Math. Soc.*, **43**, 7-60 (1938).

<sup>2</sup> If an exponential change of variable is made (3) is related to a differential operator of the type discussed by J. F. Ritt, "On a General Class of Linear Homogeneous Equations of Infinite Order with Constant Coefficients," *Trans. Am. Math. Soc.*, **18**, 27-49 (1917).

<sup>3</sup> See, for example, M. Bôcher, *Leçons sur les méthodes de Sturm*, Paris (1917), Chap. V.

<sup>4</sup> This leads to the operator  $M_{k,t}[f(x)]$  defined on p. 55 of the author's paper cited above. The operator was there seen to correspond with the kernel  $t/(x + t)^2$ .

<sup>5</sup> R. P. Boas, Jr., and D. V. Widder, "The Iterated Stieltjes Transform," *Trans. Am. Math. Soc.*, **45**, 1-72 (1939).

## THEOREMS IN THE INVERSE PROBLEM IN THE CALCULUS OF VARIATIONS

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1. *Introduction.*—In a recent issue of these PROCEEDINGS the author has announced and presented the essential features of a solution of the inverse problem of the calculus of variations recently found by him.<sup>1</sup> This problem is: *Given a curve family,  $y_i'' = F_i(x, y_j, y_j')$ , ( $i, j = 1, \dots, n$ ), in  $(n + 1)$ -dimensional space; to find, if existent, a variation problem,  $\int \varphi(x, y_j, y_j')dx = \min.$ , having this curve family as the totality of its extremals.*

A fully detailed account of our solution will appear in one of the mathematical journals. The present note summarizes the results of this detailed paper, which it is here our purpose to state.

We have given in our forthcoming paper a general method applying to an  $(n + 1)$ -dimensional space, and then carried out this plan completely

for the most important and interesting case of 3 dimensions. This results in a classification of all families  $\mathfrak{F}$  of  $\infty^4$  curves in  $xyz$ -space:

$$y'' = F(x, y, z, y', z'), \quad z'' = G(x, y, z, y', z'), \quad (1.1)$$

into extremal and non-extremal, together with a determination in the former case of the degree of generality, i.e., the number of arbitrary functions and constants, involved in the corresponding variation problem

$$\int \varphi(x, y, z, y', z') dx = \min. \quad (1.2)$$

Illustrative examples for all the more important cases are given at the end of this note. These are more completely described in our detailed paper.

In stating our results, the following notation introduced by E. Kasner in his review of Riquier's treatise on differential systems<sup>2</sup> is found useful:

$$\infty^{m_1 f(n_1) + \dots + m_k f(n_k)} \quad (1.3)$$

denotes an infinitude involving  $m_1$  arbitrary functions of  $n_1$  arguments, ...,  $m_k$  arbitrary functions of  $n_k$  arguments. This extends the classic notation  $\infty^m$  for an infinitude involving  $m$  arbitrary constants.

All functions occurring in our work are supposed to be *analytic*.

2. *The Matrix  $\Delta$ .*—We define the following symbols, functions of  $x, y, z, y', z'$ , whose values are known when the curve family  $\mathfrak{F}$  or (1.1) is given.

$$\begin{aligned} A &= \frac{d}{dx} F_{y'} - 2F_z - \frac{1}{2} F_{z'} (F_{y'} + G_{z'}), \\ B &= -\frac{d}{dx} F_{y'} + \frac{d}{dx} G_{z'} + 2(F_y - G_z) + \frac{1}{2} (F_{y'} - G_{z'}) (F_{y'} + G_{z'}), \\ C &= -\frac{d}{dx} G_{y'} + 2G_y + \frac{1}{2} G_{y'} (F_{y'} + G_{z'}). \end{aligned} \quad (2.1)$$

$d/dx$  denotes the operator

$$\frac{d}{dx} \equiv \frac{\partial}{\partial x} + y' \frac{\partial}{\partial y} + z' \frac{\partial}{\partial z} + F \frac{\partial}{\partial y'} + G \frac{\partial}{\partial z'}, \quad (2.2)$$

representing total differentiation as to  $x$  along an arbitrary curve of the given family (1.1).

From  $A, B, C$  we derive  $A_1, B_1, C_1$  by the following formulas:

$$\begin{aligned}
 A_1 &= \frac{dA}{dx} - F_{y'}A - \frac{1}{2}F_{z'}B, \\
 B_1 &= \frac{dB}{dx} - G_{y'}A - \frac{1}{2}(F_{y'} + G_{z'})B - F_{z'}C, \\
 C_1 &= \frac{dC}{dx} - \frac{1}{2}G_{y'}B - G_{z'}C;
 \end{aligned} \tag{2.3}$$

while  $A_2, B_2, C_2$  are derived from  $A_1, B_1, C_1$  by the same formulas.

Our results depend to a large extent on the rank of the matrix

$$\Delta \equiv \begin{vmatrix} A & B & C \\ A_1 & B_1 & C_1 \\ A_2 & B_2 & C_2 \end{vmatrix}. \tag{2.4}$$

For instance, it is at least a necessary condition for an extremal family that the determinant of this matrix be equal to zero. By non-satisfaction of this condition, therefore, examples of non-extremal families can be constructed at pleasure.

We begin, consequently, with a classification into cases according to the rank of  $\Delta$ , which will be followed by the appropriate sub-classifications in the statement of our theorems.

Case I:  $\begin{vmatrix} A & B & C \end{vmatrix} = 0$ ; i.e.,  $A = 0, B = 0, C = 0$ .

Case II:  $\begin{vmatrix} A & B & C \\ A_1 & B_1 & C_1 \end{vmatrix} = 0, \begin{vmatrix} A & B & C \end{vmatrix} \neq 0$ .

Case III:  $\begin{vmatrix} A & B & C \\ A_1 & B_1 & C_1 \\ A_2 & B_2 & C_2 \end{vmatrix} = 0, \begin{vmatrix} A & B & C \\ A_1 & B_1 & C_1 \end{vmatrix} \neq 0$ .

Case IV:  $\begin{vmatrix} A & B & C \\ A_1 & B_1 & C_1 \\ A_2 & B_2 & C_2 \end{vmatrix} \neq 0$ .

Here, in writing a matrix  $= 0$  we mean that each determinant resulting from it by the suppression of columns only is equal to zero, and  $\neq 0$  means that at least one such determinant is not equal to zero.

By the recursion formulas (2.3) it is seen that the cases thus described are precisely those of rank 0, 1, 2, 3 of the matrix  $\Delta$ , respectively.

3. *The Fundamental Differential System S.*—As we prove in our main paper, the solution of the inverse problem of the calculus of variations for the given curve family (1.1) is equivalent exactly to the solution of the following linear differential system,  $S$ , for the unknown functions  $L, M, N$  of  $x, y, z, y', z'$ :

$$\frac{dL}{dx} + F_y L + G_y M = 0,$$

$$\frac{dM}{dx} + \frac{1}{2}F_z L + \frac{1}{2}(F_y + G_z)M + \frac{1}{2}G_y N = 0,$$

$$\frac{dN}{dx} + F_z M + G_z N = 0;$$

$$AL + BM + CN = 0;$$

$$L_z = M_y, N_y = M_z;$$

$$LN - M^2 \neq 0. \quad (3.1)$$

4. *The "Critical Cone."*—The *inequation* (3.1), last relation of the system  $S$ , is very important. Its negation,

$$LN - M^2 = 0, \quad (4.1)$$

defines a quadric cone in an auxiliary  $LMN$ -space, whose significance first appears in our work on the inverse problem, and which we call the "*critical cone*," denoting it by  $\mathfrak{R}$ .

The purely algebraic equation (3.1) of the differential system,  $S$ :

$$AL + BM + CN = 0, \quad (4.2)$$

defines a plane  $\mathfrak{P}$  in the  $LMN$ -space passing through the vertex of the critical cone, located at the origin. In the discussion of Case II—which is the most interesting and varied in its results—much depends on whether the plane  $\mathfrak{P}$  intersects the critical cone  $\mathfrak{R}$  in two distinct generators (real or conjugate imaginary) or, on the other hand, is tangent to this cone.

The intersection of  $\mathfrak{P}$  with  $\mathfrak{R}$  is determined by the quadratic equation

$$A\xi^2 + B\xi + C = 0, \quad (4.3)$$

whose roots, known functions of  $x, y, z, y', z'$ , will be denoted by  $\lambda, \mu$ . Accordingly, we make the following subdivision of Case II:

Case IIa:  $B^2 - 4AC \neq 0$ , or  $\lambda \neq \mu$ ;

Case IIb:  $B^2 - 4AC = 0$ , or  $\lambda = \mu$ .

In Case IIa the following symbols intervene in the statement of our results, where the importance of the hypothesis  $\lambda \neq \mu$  is seen in the presence of  $\lambda - \mu$  in various denominators.



$$\begin{aligned}
\alpha &= \frac{\lambda\lambda_{z'} - \lambda_{y'}}{\lambda - \mu}, \quad \beta = \frac{\mu\mu_{z'} - \mu_{y'}}{\lambda - \mu}, \\
H &= \frac{1}{2} F_{z'} \lambda^2 - \frac{1}{2} (F_{y'} - G_{z'}) \lambda - \frac{1}{2} G_{y'}, \\
I &= \lambda_z + H_{z'} - R, \\
J &= \frac{(H - P)\beta + K}{\lambda - \mu}, \\
K &= \mu\mu_z - \mu_y + P\mu_{z'} - \mu P_{z'} + P_{y'}, \\
P &= \frac{1}{2} F_{z'} \mu^2 - \frac{1}{2} (F_{y'} - G_{z'}) \mu - \frac{1}{2} G_{y'}, \\
Q &= \mu_z + P_{z'} - J, \\
R &= -\frac{(H - P)\alpha + S}{\lambda - \mu}, \\
S &= \lambda\lambda_z - \lambda_y + H\lambda_{z'} - \lambda H_{z'} + H_{y'}.
\end{aligned} \tag{4.4}$$

In Case IIb the following symbols are important:<sup>3</sup>

$$\begin{aligned}
\text{(I)} &= \frac{1}{2} F_{z'} \lambda^2 - \frac{1}{2} (F_{y'} - G_{z'}) \lambda - \frac{1}{2} G_{y'}, \\
\text{(II)} &= F_{z'} \lambda - \frac{1}{2} (F_{y'} - G_{z'}), \\
\text{(III)} &= \lambda_z + \text{(I)}_{z'}, \\
\text{(IV)} &= \text{(II)}_{z'}, \\
\text{(V)} &= \lambda\lambda_{z'} - \lambda_{y'}, \\
\text{(VI)} &= \lambda\lambda_z - \lambda_y + \text{(I)}\lambda_{z'} - \lambda\text{(I)}_{z'} + \text{(I)}_{y'} + \text{(II)}\text{(V)}, \\
\text{(VII)} &= 2\lambda_z + 2\lambda_{z'}\text{(II)} - \lambda\text{(II)}_{z'} + \text{(II)}_{y'}, \\
\text{(VIII)} &= \lambda\text{(VI)}_{z'} - \text{(VI)}_{y'} + \text{(V)}_y - \lambda\text{(V)}_z + \lambda_{z'}\text{(VI)} - \text{(I)}\text{(V)}_{z'} + \\
&\quad \text{(III)}\text{(V)} - \text{(V)}\text{(VII)}, \\
\text{(IX)} &= \lambda\text{(VII)}_{z'} - \text{(VII)}_{y'} - 2\lambda_{z'z'}\text{(I)} + 2\lambda_{yz'} - 2\lambda\lambda_{zz'} + \\
&\quad \text{(IV)}\text{(V)}.
\end{aligned} \tag{4.5}$$

Also, we denote by (VI'), (IX') the expressions (VI), (IX) without their respective last terms.

5. *Statement of Results.*—THEOREM I. *Every curve family  $\mathfrak{F}$  which obeys the conditions  $A = 0$ ,  $B = 0$ ,  $C = 0$  of Case I is an extremal family, and the*

generality of the corresponding variation problem is expressed by the symbol  $\infty^{2f(3)+2f(2)}$ .

It should be stated here that, in counting the arbitrary functions involved in the determination of  $\varphi$  when  $F, G$  are given, we omit the function  $\nu(x, y, z)$  in the arbitrary exact differential  $d\nu(x, y, z)$  that may always be added to  $\varphi dx$  without changing the extremals.

THEOREM II. In Case IIa, if the conditions

$$\alpha = 0, \beta = 0, S = 0, K = 0 \quad (5.1)$$

are verified, then the curve family  $\mathfrak{F}$  is one of extremals, and the generality of the corresponding variation problem is  $\infty^{2f(2)}$ .

THEOREM III. In Case IIa, if the conditions (5.1) are not verified, but certain other conditions (stated in our forthcoming complete paper) are, then the given curve family  $\mathfrak{F}$  is of extremal nature and belongs to  $\infty^{1f(2)+1f(1)}$  different variation problems.

THEOREM IV. In Case IIb, if the conditions

$$(V) = 0, (VI') = 0, (IX') = 0 \quad (5.2)$$

are satisfied, then the given curve family  $\mathfrak{F}$  can be identified with the extremals of a class of variation problems whose generality is  $\infty^{2f(2)}$ .

As a preliminary to the statement of our next theorems, we introduce symbols to represent the second order determinants contained in the two-by-three matrix whose non-vanishing figures in Case III, namely:

$$\Delta_1 = BC_1 - B_1C, \Delta_2 = CA_1 - C_1A, \Delta_3 = AB_1 - A_1B. \quad (5.3)$$

Also, we let

$$D = \Delta_1\Delta_3 - \Delta_2^2. \quad (5.4)$$

Making then in the differential system,  $S$ , or (3.1), the substitutions

$$L = \rho\Delta_1, M = \rho\Delta_2, N = \rho\Delta_3, \quad (5.5)$$

we obtain a differential system of the following simple form for  $\rho(x, y, z, y', z')$  as unknown function:

$$\rho_x = E_1\rho, \rho_y = E_2\rho, \rho_z = E_3\rho, \rho_{y'} = E_4\rho, \rho_{z'} = E_5\rho, \rho \neq 0; \quad (5.6)$$

where the  $E_i$  are rational expressions in the partial derivatives of  $F, G$  involving only  $D$  as denominator, and therefore existing as calculable known functions if  $D \neq 0$ .

THEOREM V. In Case III, if  $D = 0$ , then the given curve family is non-extremal.

THEOREM VI. In Case III, if  $D \neq 0$ , then a necessary and sufficient condition for the given curve family  $\mathfrak{F}$  to be one of extremals is that the differential

$$E_1dx + E_2dy + E_3dz + E_4dy' + E_5dz' \quad (5.7)$$

be exact. The corresponding variation problem is essentially uniquely determined, i.e., up to a constant multiplier and an arbitrary additive exact differential,  $dv(x, y, z)$ .

THEOREM VII. All curve families  $\mathfrak{F}$  in Case IV are non-extremal.

The preceding theorems cover all the more interesting and important cases. Certain additional minor cases are discussed in our detailed paper.

6. *Examples.*—THEOREM I:  $y'' = f(z')$ ,  $z'' = 0$ , where  $f$  denotes an arbitrary function. This includes the case of the straight lines:  $y'' = 0$ ,  $z'' = 0$  treated by G. Hamel in a well-known paper.<sup>4</sup>

THEOREM II: The "separated case:"  $y'' = F(x, y, y')$ ,  $z'' = G(x, z, z')$ ,  $B \neq 0$ .

THEOREM III: The  $\infty^4$  catenaries which lie in planes perpendicular to the  $xz$ -plane and the directrix of each of which coincides with the trace of its plane upon the  $xz$ -plane:

$$y'' = \frac{1 + y'^2 + z'^2}{y}, \quad z'' = 0.$$

THEOREM IV:  $y'' = z$ ,  $z'' = 0$ .

THEOREM V:  $y'' = y^2 + z^2$ ,  $z'' = 0$ .

THEOREM VI:  $y'' = z^2$ ,  $z'' = y^2$ . The corresponding variation problem is:  $\int \left( y'z' + \frac{1}{3}y^3 + \frac{1}{3}z^3 \right) dx = \min.$ , up to the slight possibility of modification expressed in Theorem VI.

THEOREM VII:  $y'' = y^2 + z^2$ ,  $z'' = y$ .

<sup>1</sup> J. Douglas, "Solution of the Inverse Problem of the Calculus of Variations," these PROCEEDINGS, 25, 631-637 (Dec., 1939).

<sup>2</sup> *Bull. Amer. Math. Soc.*, 19, 14 (1913).

<sup>3</sup> Subscripts attached to the roman numerals in parentheses denote partial differentiation.

<sup>4</sup> "Über die Geometrien in denen die Geraden die Kürzesten sind," *Math. Annalen*, 57, 231-264 (1903).



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*CYTOPLASMIC BEHAVIOR DURING DIVISION OF VACUOLATE  
PLANT CELLS*

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The factors which determine the plane in which a meristematic cell divides must evidently be very important in controlling direction of growth and thus the development of form. In plant cells the orientation of the chromatic material, and particularly the distribution of the chromosomes at metaphase, give an indication as to where the plane of nuclear division is to be. Little evidence has been obtained, however, from the structure of either nucleus or cytoplasm in early mitosis which indicates where the cell itself is to divide, although Bowen<sup>1</sup> and others have shown that in certain cells the chondriosomes assume a characteristic position with reference to the future axis of the mitotic figure. Most of the plant cells in which mitosis has been studied are relatively small ones, which are rich in cytoplasm, and in which large vacuoles are absent. Cells which are larger and strongly vacuolate and yet are still dividing, and which in the aggregate are perhaps even more numerous than the more "typically" meristematic cells, have been largely neglected by cytologists. A study of cytokinesis in such cells makes it clear that the plane of the next division, and indeed the exact location of the future cell wall, are indicated by the distribution of the cytoplasm at a stage much earlier than one where these facts can be determined from nuclear orientation.

In such studies it is evidently necessary to observe cells in which the divisions are all in the same plane and in which the position of each new wall can therefore be predicted with some certainty. Such cells may be found in various parts of the plant, notably in the "rib" meristems of the young pith and cortex of the stem, where the divisions are all transverse to the axis. Even more favorable material is provided by the secondary meristems which are induced in fundamental tissue as a result of wounding. Here the new walls are all essentially parallel to the wound surface, and there is the added advantage that in the early divisions, at least, these new

division walls in adjacent cells are usually directly opposite each other, so that the future position of the wall in a dividing cell may often be predicted very definitely. The cells are also relatively much larger than in

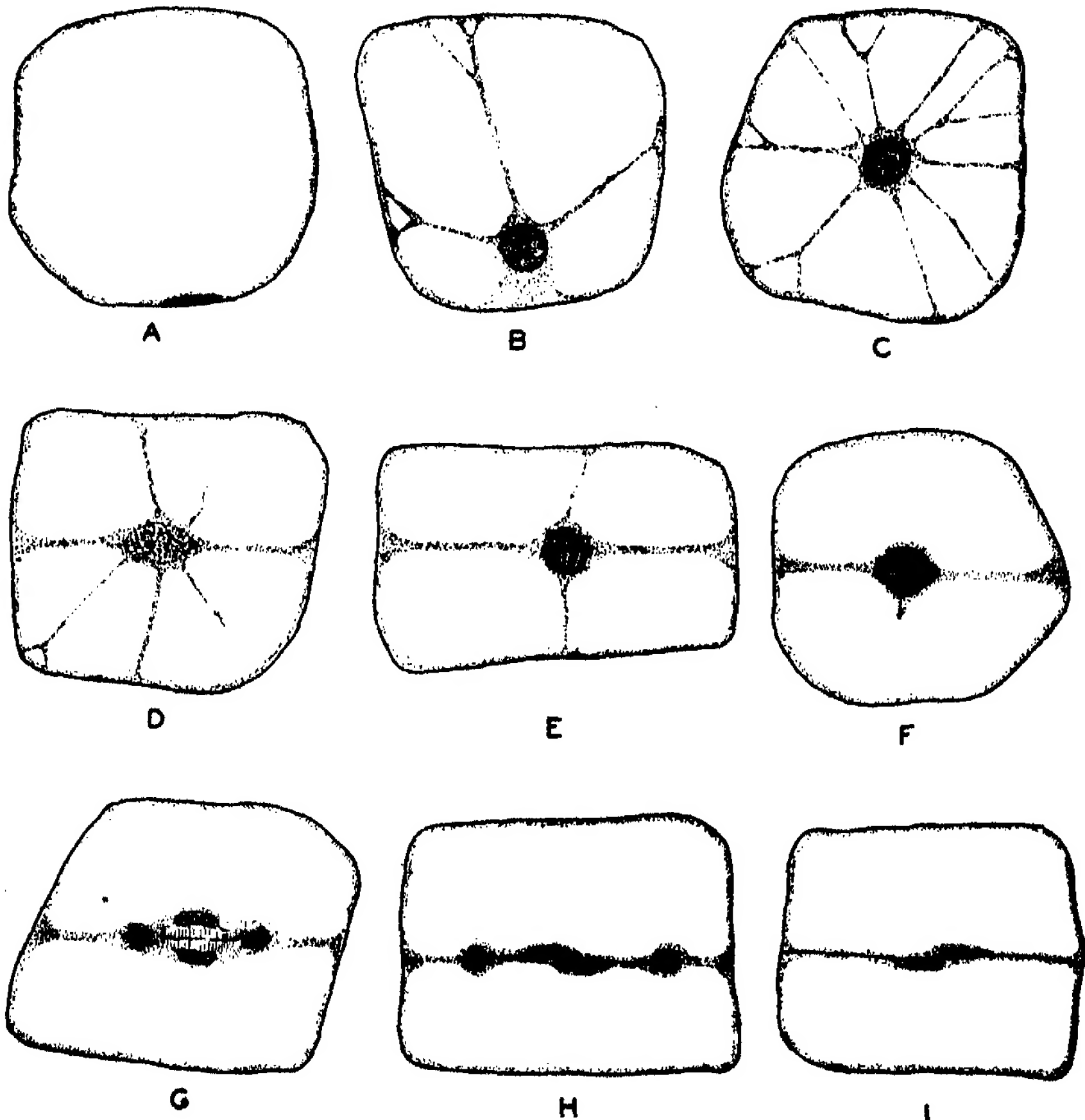


FIGURE 1

Semi-diagrammatic drawings of cell division in pith cells of *Ricinus*, which has been induced by wounding. The wound surface is parallel to the bottom of the page. *A*, resting stage; *B*, early prophase, enlarged nucleus migrating toward center of cell; *C*, prophase, showing beginning of formation of equatorial plasma strands; *D*, late prophase, with well developed phragmosome; *E*, metaphase; *F*, anaphase; *G* and *H*, telophases, showing development of young wall which follows the course of the phragmosome; *I*, two complete daughter cells.

ordinary meristematic tissue, since fully grown cells become dedifferentiated and meristematic, and the processes of cell division, particularly cytokinesis, may thus be seen in them very clearly.



Transverse, radial and tangential sections through wound tissues in a number of plants were studied. In figure 1, *A-I*, are shown semi-diagrammatic drawings of nine stages in the division of large pith cells in *Ricinus* which had been induced to divide by wound stimulus. In every case the wound face, and thus the future direction of the wall, is parallel to the bottom of the page.

In *A* is shown a typical differentiated cell, with the nucleus greatly flattened against the wall, cytoplasm small in amount and the bulk of the cell consisting of a large vacuole. The first effect of the wound stimulus, shown in *B*, is an increase in amount of cytoplasm, a rounding up and enlargement of the nucleus, and its ascent, on a column of cytoplasm, toward the center of the vacuole. Strands of cytoplasm begin to be thrown out to the wall. In *C*, the nucleus is now suspended in the vacuole by strands of cytoplasm. These are for the most part random in direction, but, from the very beginning of this stage, some are always present in the plane of the future wall. In *D*, a later prophase, these equatorial strands have become much heavier than the others and are now fusing at their bases. The nucleus is no longer spherical but is somewhat extended in the plane of its future division. Often at this stage there is one cytoplasmic strand passing upward and another downward at right angles to the plane of future division. *E* is the metaphase and *F* the anaphase of division, the nucleus now being suspended chiefly by the rather heavy cytoplasmic diaphragm which marks the position of the future wall. Other strands have often entirely disappeared at this stage. In *G*, the phragmoplast at telophase is extending laterally, the cell plate being carried far beyond the limits of the original nucleus by kinoplasmic fibrils, in the manner described by Strasburger, Treub, Bailey and others,<sup>2</sup> the system in face view giving the appearance of a circle or "halo." It is significant that the course of the kinoplasmosome follows the equatorial diaphragm of cytoplasm established from early prophase, and that the developing cell wall which is being laid down in this way, and which finally reaches the wall of the mother cell, thus coincides in its position with the cytoplasmic plate. Before this process is completed the daughter nuclei (*H*) have usually returned to the resting condition. In *I* are shown the two daughter cells with the new wall between them, which occupies the position determined by the strands of cytoplasm very early in division. All the cells figured show an early division after wounding, and later cells are necessarily smaller, but they divide in the same way.

An essentially similar cycle of mitotic changes was observed in wounded tissues of *Tradescantia*, *Kalanchoe*, *Bryophyllum*, *Coleus*, *Phaseolus*, *Petunia* and *Cucurbita*. The results were entirely confirmed by direct observation of living dividing cells in hand sections from wounded regions.

The distinctive feature of the mitotic process in vacuolate cells, where

the amount of cytoplasm is necessarily small in proportion to the size of the cell, is that this cytoplasm, from a very early stage in division, tends to become aggregated into a series of strands, sometimes anastomosing into a diaphragm, which occupies the position of the future wall and which thus indicates, considerably before the nucleus has done so, where the plane of division is to be. For this plate of cytoplasm the writers propose the term *phragmosome*.

That the phragmosome maintains its original position from the earliest stages to final wall formation is indicated by the fact that in early divisions of wounded tissue, where the new walls form a continuous series so that the wall of one cell is exactly opposite that of the next, the phragmosome is formed in the plane where the wall must ultimately be laid down. Evidence from plasmolyzed cells also shows that the phragmosome is firmly attached to the wall.

A number of minor differences from the method here described may sometimes be observed. Thus in many cases the nucleus remains at or near one wall during division, and then the phragmosome and the wall which follows it are formed on only one side of the nucleus. Division is usually approximately equal, but it frequently happens that the nucleus and phragmosome take up a position nearer one end of the cell than the other, so that the two daughter cells are markedly unequal. The phragmosome and wall commonly lie straight across the cell but occasionally, especially when division is unequal or the nucleus lies near one corner, the partition may be curved from the start.

Normal meristematic tissues, especially at the tip of stem and root, in which mitosis has chiefly been studied, are not favorable material for observation of the phragmosome, since the cells and vacuoles are small. In tissues where the cells are still dividing but where rather large vacuoles have already appeared, as in regions some distance back from the growing point in the developing pith and cortex, or in the fundamental tissue of massive organs like the fruit, the plate of cytoplasm may readily be seen, and such cells divide in essentially the same manner as has been described for wound meristems. The phragmosome thus seems to be a characteristic feature of the division of cells which have rather large vacuoles, and a visible expression of the polarity of such cells.

The significance of the phragmosome lies chiefly in its indication that the factors which determine the plane of division of the cell act upon the cell as a whole and not upon the nucleus alone. From very early prophase the position of the new wall is visibly determined in the cytoplasm. Nuclear orientation may not at first agree with this cytoplasmic orientation, for the equatorial plane of the figure often fails to lie parallel with the phragmosome; and it is thus evident, as has frequently been observed, that the figure is moving or rolling about in the cytoplasm. At telophase,

however, it always comes back to a position where the cell plate is parallel to the phragmosome. All this suggests that the establishment of the division plane may be effected first in the cytoplasm rather than in the nucleus. The phragmosome is also of significance in those problems which deal with the factors determining the relative position of cell walls in multicellular tissues. The question, for example, as to whether the new walls are oriented as liquid films would be, in response to surface tension, or whether quite different factors are here involved, will require a study of the forces acting upon the phragmosome from the beginning and not alone upon the growing wall which follows it. A function of the phragmosome is presumably that of providing the material from which the new wall is built, and it is therefore easy to understand why the developing wall should follow it so closely.

The failure of cytologists to recognize such a conspicuous structure as the phragmosome is probably due to the fact that students of cell division have concerned themselves almost entirely with small cells having few or very small vacuoles. A recognition of the presence of such a structure occurs in two papers by Hanstein<sup>3,4</sup> in 1870 and 1880 which have been generally overlooked by later workers. Hanstein studied cell division in vacuolate dividing cells of the pith of various plants, and he figured and briefly mentioned a plate of cytoplasm in the plane of cell division, but failed to realize its significance for cytology and development.

*Summary.*—A study of cell division in vacuolate plant cells shows that from very early prophase the cytoplasm tends to become aggregated into a plate of more or less fused strands, the phragmosome, which occupies the position where the future cell wall will be formed. The fact that the entire cell body rather than the nucleus alone appears to be concerned in establishing the plane of division and the location of the new wall in plant cells is of general importance in problems of development.

<sup>1</sup> R. H. Bowen, *La Cellule*, 39, 123-156 (1929).

<sup>2</sup> Described and literature cited in G. Tischler, *Allgemeine Pflanzenkaryologie*, Berlin (1921-1922).

<sup>3</sup> J. Hanstein, *Sitzungsber. Niederrhein. Ges. Natur- und Heilkunde Bonn*, Sitz., 19 Dec. 1870. (Reprinted in *Bot. Zeit.*, 30, 22-28, 41-46 (1872).

<sup>4</sup> J. Hanstein, *Bot. Abhandlungen*, 4 (2), 1-56 (1880).

## FURTHER OBSERVATIONS ON THE MECHANISM OF INDUCED CHROMOSOME REARRANGEMENT IN *SCIARA*

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It has been shown in an earlier paper (by Metz and Boche)<sup>1</sup> that in oöcytes of adult females of *Sciara* the chromosomes appear to be very "resistant" to irradiation, as judged by the difficulty of inducing chromosome rearrangements. Whereas, in sperms rearrangements were readily obtained through irradiation, few if any rearrangements were secured by treating eggs. Since this result contrasted with those recorded from work on *Drosophila* it was suggested that the difference may be due to differences in mode of development of the oöcytes in the two genera. In *Drosophila* all stages from oögonia to mature eggs may be found in one ovary, while in *Sciara* the oöcytes develop synchronously and only relatively old oöcytes are present in adult females. Preliminary observations on the chromosomes of the latter suggested that at the time of treatment the chromosomes were in a condensed condition ready or nearly ready for mitosis and that their physical condition at this time made them resistant to irradiation.

The present account deals with an extension of the study, which leads to a different interpretation from that suggested previously. The results indicate that the chromosomes are "resistant" to irradiation for a much longer period than was formerly supposed, including a stage in which they are long and thread-like, and that it is probably lack of movement, rather than any special characteristic of the chromosomes themselves, which is responsible for the absence of chromosome rearrangements.

One of the primary obstacles encountered in the earlier work was the difficulty of securing good cytological material, due to the well-known refractoriness of insect eggs. This difficulty has now been overcome to a considerable extent by the development of a new technique which makes possible a detailed study of the oöcyte chromosomes. In the new procedure the nucleus (germinal vesicle) is removed from the egg and exposed directly to the fixative in a smear—thus securing almost instantaneous fixation and avoiding the processes of embedding and sectioning. This involves treating each egg separately, breaking it open and spreading the contents enclosing the nucleus with a rapid stroke of the needle, and applying the fixative before the material has had a chance to dry. The present observations were made on such material, supplemented by whole mounts prepared by the Feulgen method formerly used for study of maturation and cleavage stages.<sup>2</sup>

Attention has thus far been given mainly to eggs of females less than ten



hours old. At this time the eggs are more favorable for study than they are later. Furthermore, it is known that this is a period during which the chromosomes are "resistant" to irradiation.<sup>3</sup> During the period in question the oöcyte chromosomes are in a typical early to middle prophase condition. The tetrads are long slender threads, fairly uniformly spaced, extending about the nucleus near the periphery.<sup>4</sup>

These findings seem to indicate that in the oöcytes of *Sciara* typical prophase chromosomes are "resistant" to irradiation. Such a result stands in contrast to those reported from other organisms, in which the prophase, including the meiotic prophase, is one of the "sensitive" stages.<sup>5</sup> The apparent difference in susceptibility is difficult to explain. It is evidently not due, as previously suggested, to a condensed condition of the chromosomes in *Sciara*. Neither does it seem to be due to the distance between the chromosomes. Measurements on fixed material indicate that the chromosome threads in the present case lie approximately one to two micra apart, with some regions occasionally even closer together. It seems improbable that these distances are greater than are those at comparable stages in such organisms as *Tradescantia* where rearrangements are readily secured.

Assuming that irradiation breaks the chromosome threads in *Sciara* as it appears to do in other organisms, the only plausible explanation for our results would seem to be that the broken ends fail to move enough to form new combinations. Some evidence on this point has been secured by cytological examination of the chromosomes during and subsequent to treatment. It is well known that one of the common effects of irradiation is a clumping of the chromosomes. The material was examined, therefore, for evidence of clumping, which would, of course, be evidence of movement. At the same time the chromosomes were examined for evidence of visible breaks.

For technical reasons radium was used as an agent<sup>6</sup> in this part of the investigation, and pupae instead of adults were treated. The radium dosage<sup>7</sup> is known from other tests to be effective in inducing rearrangements in sperms, and the chromosomes in late pupal oöcytes are known to be similar in appearance to those of young adults (R. O. Berry, unpublished).

Seventy-one nuclei were examined from preparations made during the treatment, after receiving from 3 to 4 gram hours.<sup>7</sup> From each pupa two to four preparations were made. Fixation was effected in the first within five minutes, and in the last within fifteen minutes, after removal from the radium. In addition seventeen nuclei were examined from preparations made in the same way at the end of 30 minutes, and twenty-one nuclei from others made one hour after completion of the four gram hour treatment.

The results of these observations may be summarized by saying that no

significant evidence of clumping or of breakage of chromosomes was detected. The chromosomes could not be distinguished from those in untreated material.<sup>8</sup>

Further data will be required before final conclusions can be drawn respecting this point, but the evidence suggests that lack of chromosome movement is the main factor in preventing chromosome rearrangement here. If this is the case it is possible that rearrangements may be induced by rayed eggs during the meiotic divisions, which probably occur at a later stage than any of those thus far used in our irradiation work. Such an interpretation may help to explain the findings of Patterson,<sup>9</sup> who reports in *Drosophila* an increase in the frequency of rearrangements in old, as compared with young eggs. Experiments have been planned to test the viscosity of the nuclear contents during the stages under consideration in *Sciara* in the hope that this may also throw light on the question of movement. As already noted, the "prophase" condition of the chromosomes persists here for a long time (probably three or four days or more) and there is presumably little movement during that period. The length of the period does not seem to be the significant factor, however, for in *Tradescantia*, where chromosome rearrangements are readily induced during prophase, the period is also of long duration (Sax).<sup>5</sup> It seems probable that in the latter material irradiation acts on the nuclear constituents (by reducing the viscosity?) in such a way as to bring about clumping of the chromosomes, whereas in *Sciara* oöcytes it does not.

As mentioned in the earlier paper,<sup>1</sup> it might be suggested that rearrangements occur in the *Sciara* eggs, but are not recovered in the offspring because the modified chromosomes are eliminated at meiosis. Such an explanation not only seems improbable, but is opposed by our present observations. Examination of the oöcyte chromosomes after irradiation has revealed no evidence of rearrangements. This is probably not due to difficulty of detecting rearrangements, for in oöcytes carrying chromosomes from rayed sperms rearrangements have been observed.

Further comparison of our results on *Sciara* with those of other investigators on *Drosophila* is difficult at present because of the uncertainty as to what happens in irradiated oöcytes of *Drosophila*. There appeared at first to be a wide divergence between conditions in the two genera, but the recent observations of Glass<sup>10</sup> in which he found no translocations following irradiation of *Drosophila* oöcytes, suggest that "sensitivity" in *Drosophila* may not be as different from that in *Sciara* as the earlier records would indicate.

No attempt will be made here to discuss the possible significance of our failure to find direct evidence of chromosome breaks in the treated oöcytes. The result might be considered as favoring the "contact" hypothesis of chromosome rearrangement rather than that which assumes a breakage of



the chromosome threads followed by movement and subsequent union of free broken ends. On the other hand, however, the result may also be explained by assuming that only one or two chromatids of the tetrad are broken at any one point and that consequently the breaks are not visible, or that, with lack of movement, the broken ends quickly become re-attached.

<sup>1</sup> Metz, C. W., and Boche, R. D., these PROCEEDINGS, 25, 280 (1939).

<sup>2</sup> Schmuck, Louise M., and Metz, C. W., *Science*, 74, 600 (1931).

<sup>3</sup> This is shown by the earlier data, which came largely from females treated when less than twelve hours old, supported by more recent experiments of Metz and Lane (unpublished). In the latter, virgin females were treated at various ages (each receiving 5000 r of x-rays) and the salivary gland chromosomes of the  $F_1$  larvae examined for rearrangements. From females treated when less than three hours old 27 specimens were examined; from females treated at between 4 and 23 hours 12 were examined. No rearrangements were found.

<sup>4</sup> Their appearance in the sectioned material previously used was presumably due to shrinkage of the nuclear contents during fixation.

<sup>5</sup> See, e.g., Sax, K., *Genetics*, 23, 494 (1938) and *Ibid.*, 25, 41 (1940).

<sup>6</sup> The radium treatments were given by Dr. Fred West, of the Kelly Hospital, Baltimore, and the x-ray treatments by Dr. Louis B. Maxwell of the Bureau of Chemistry and Soils, U. S. Department of Agriculture, to both of whom we express especial appreciation.

<sup>7</sup> Three to four gram hours at 1 inch, through 2 mm. of brass, given within less than four hours. Preliminary experiments on males indicate that this is probably as effective as 5000 r of x-rays.

<sup>8</sup> It may be added that the salivary gland chromosomes have been examined from 49  $F_1$  larvae from females which had been given 5000 r units of x-rays or 4 gram hours of radium during the pupal stage. Only one exhibited a rearrangement (a translocation).

<sup>9</sup> Patterson, J. T., *Genetics*, 18, 32 (1933).

<sup>10</sup> Glass, H. B., *Genetics*, 25, 117 (1940).

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## CONJUGATION OF THREE ANIMALS IN *PARAMECIUM BURSARIA*

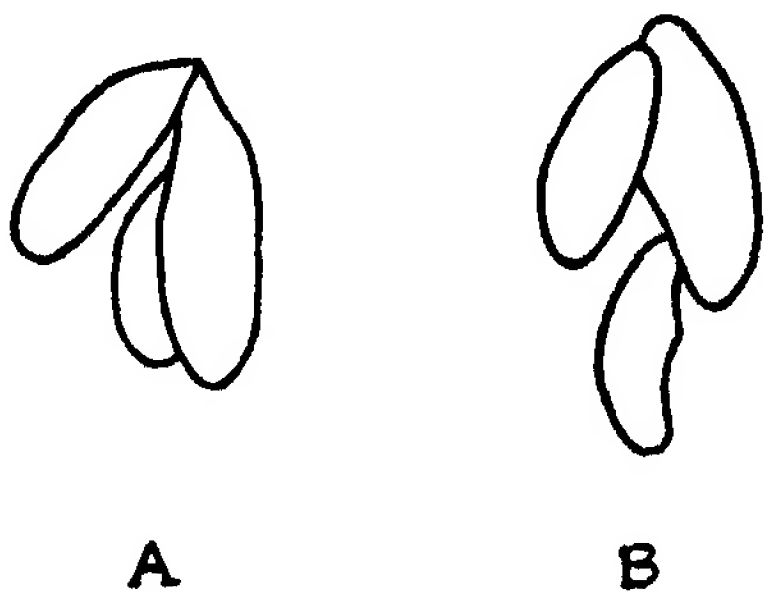
BY TZE-TUAN CHEN<sup>1</sup>

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Communicated March 6, 1940

In *Paramecium bursaria*, in addition to the usual pairs, three animals may conjugate.<sup>2</sup> Nuclear changes occur in all of the three conjugants. Conjugation of three animals in *P. bursaria* is of more than cytological interest because it throws light upon some physiological aspects of conjugation in *Paramecium*.

The present study was made chiefly on two races of *P. bursaria*—*McD*<sub>3</sub> and *Fd*—although three animals in conjugation have also been observed in matings of a number of other races of this species.<sup>3</sup> These two races (*McD*<sub>3</sub> and *Fd*) which belong to two different mating types in group II are particularly favorable for the present study because of the regular, marked differences in the size and in the staining capacity of the micronucleus. *McD*<sub>3</sub> (collected from the vicinity of Baltimore) has a relatively large micronucleus which stains deeply with haematoxylin and contains a relatively large quantity of chromatin (Fig. 1). The pronuclei of this race are also large and stain deeply (Fig. 7). *Fd* (collected also from the vicinity of Baltimore) has a relatively small micronucleus which stains rather lightly and contains a small quantity of chromatin (Fig. 2). The pronuclei in this race are also small and stain lightly (Fig. 7). Because of these differences between the two races all of the three conjugants can be easily identified. It is also possible to ascertain whether the third conjugant is



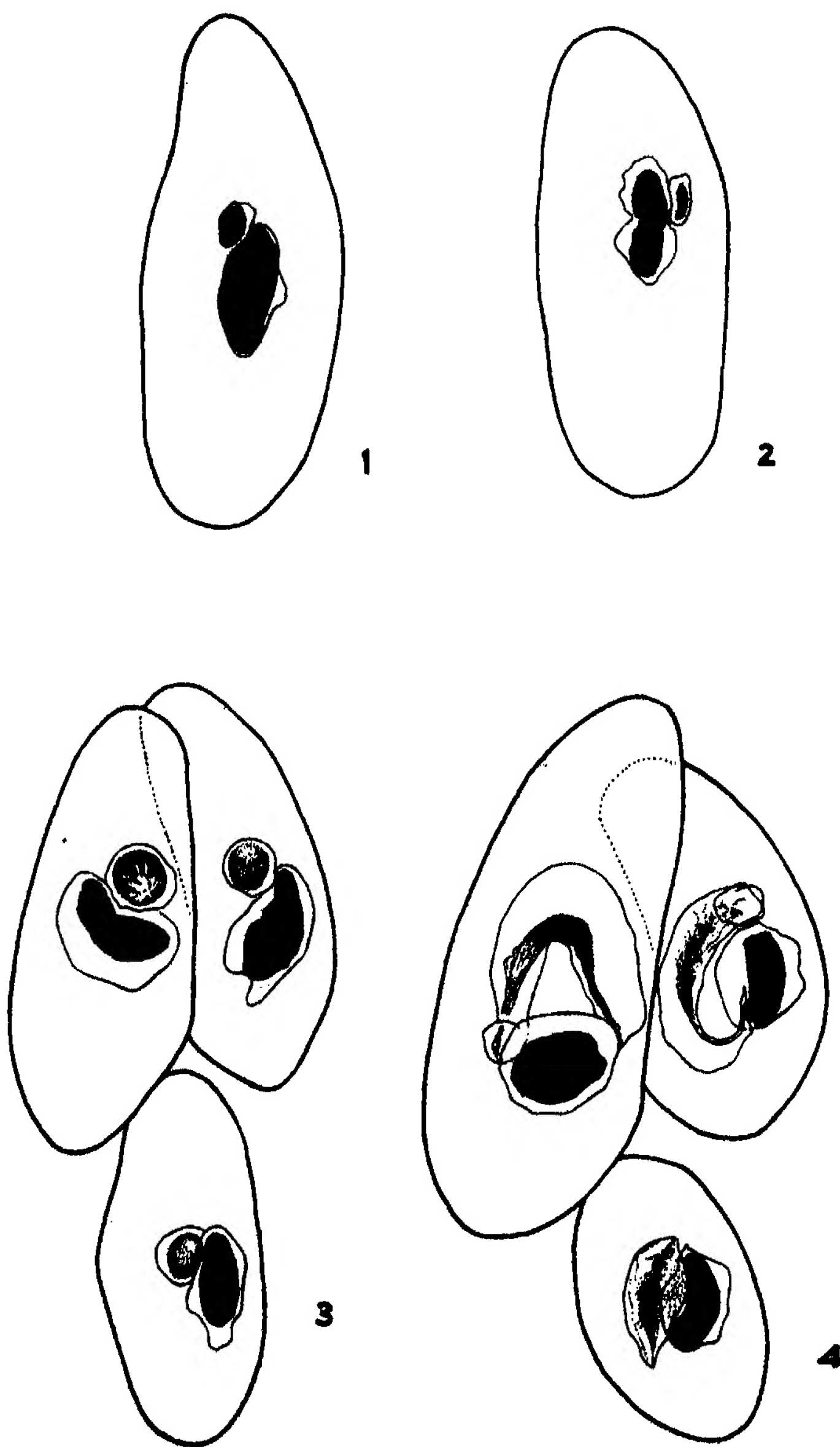
FIGURES A AND B

Two types of association of three conjugants.

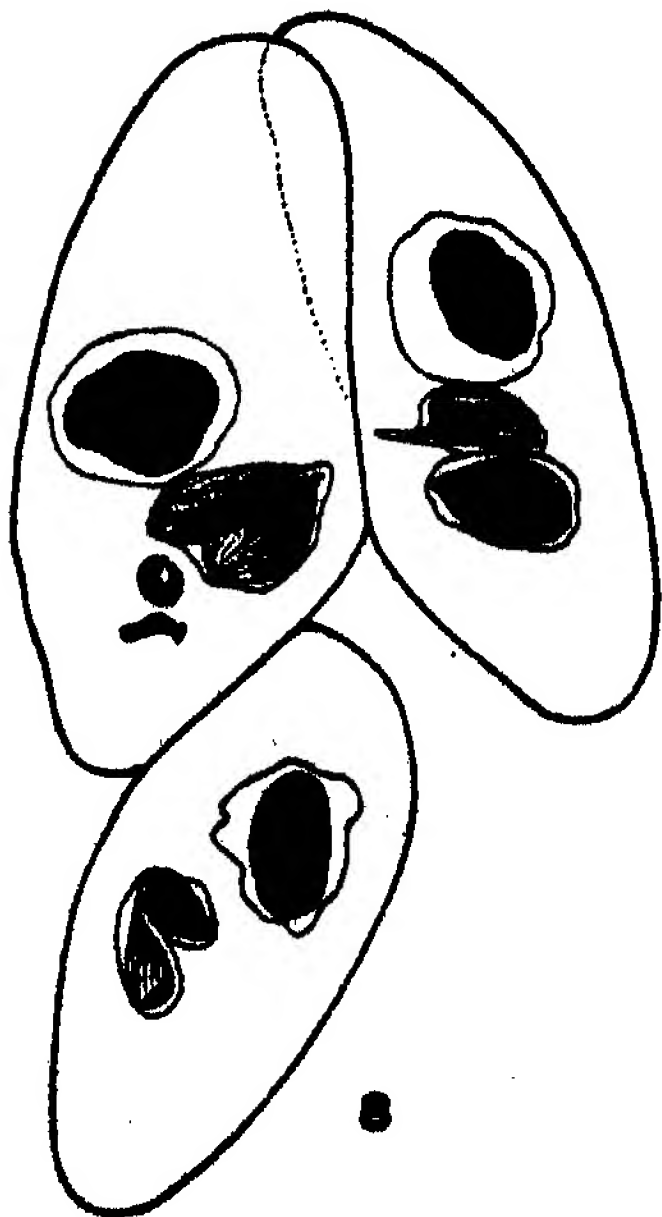
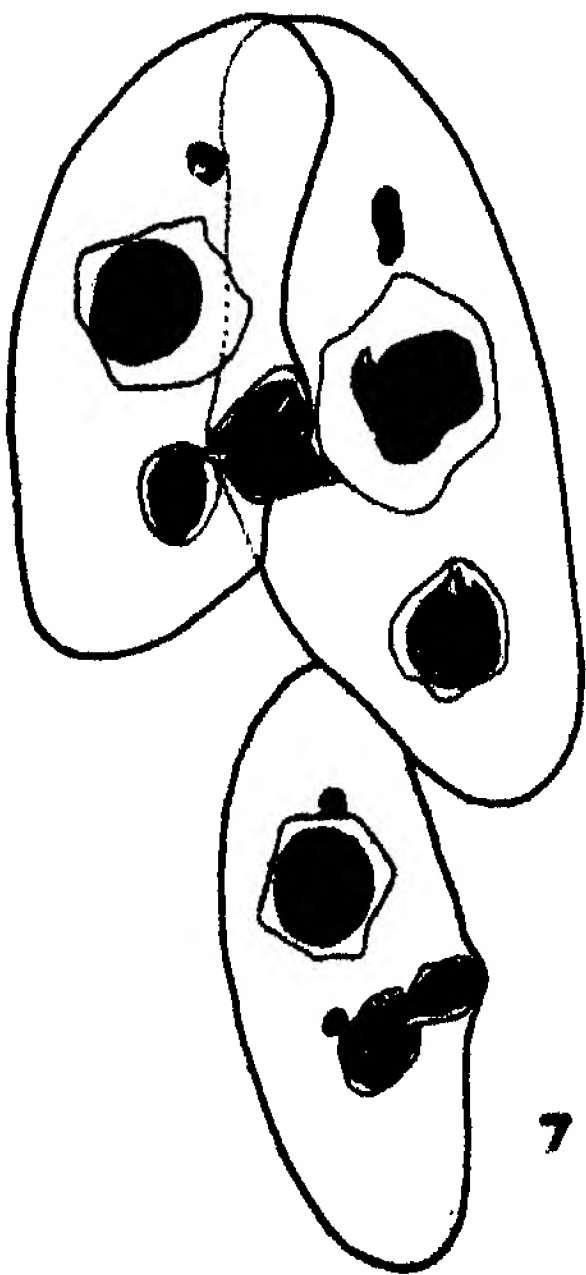
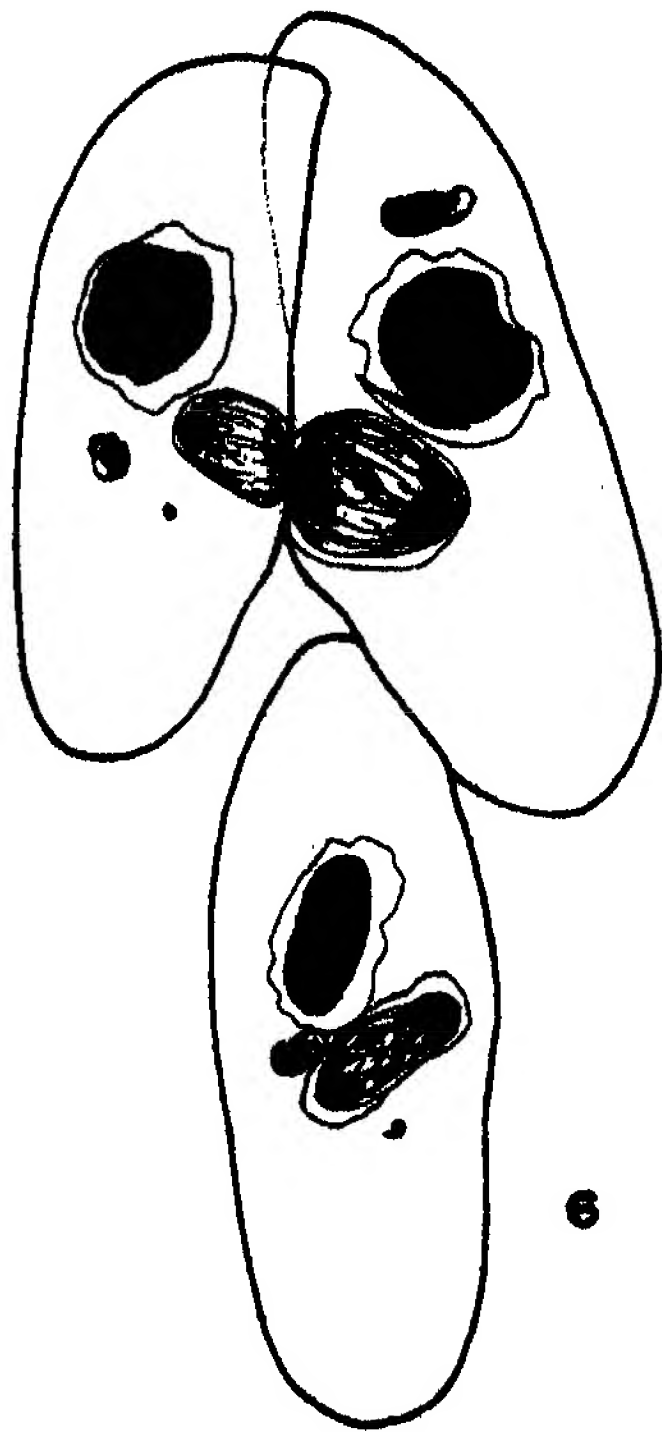
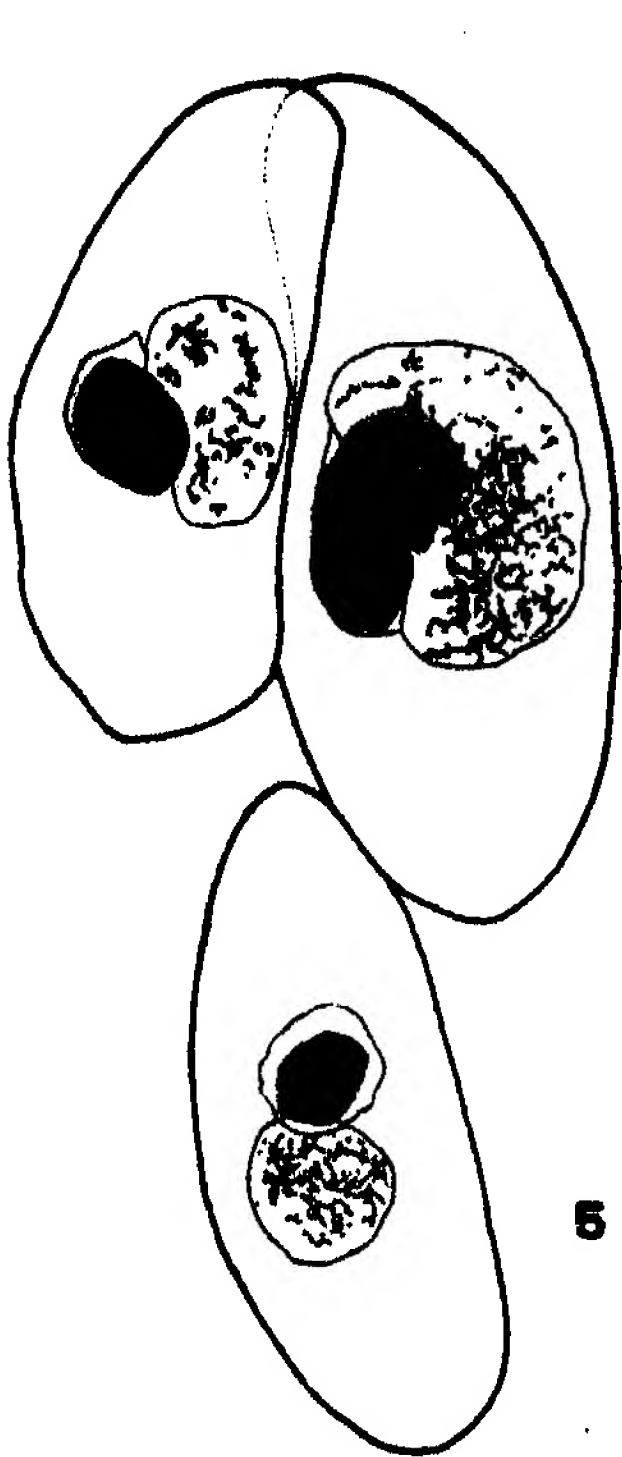
attached to a conjugant of the same race or to a conjugant belonging to the other race, and to ascertain whether an exchange of pronuclei occurs between the third conjugant and the conjugant to which it is attached.

Under appropriate conditions, animals belonging to these two races will, when they are mixed, immediately agglutinate and soon form pairs. In addition to the usual pairs, three animals in conjugation have been regularly found in mixtures of these two races.

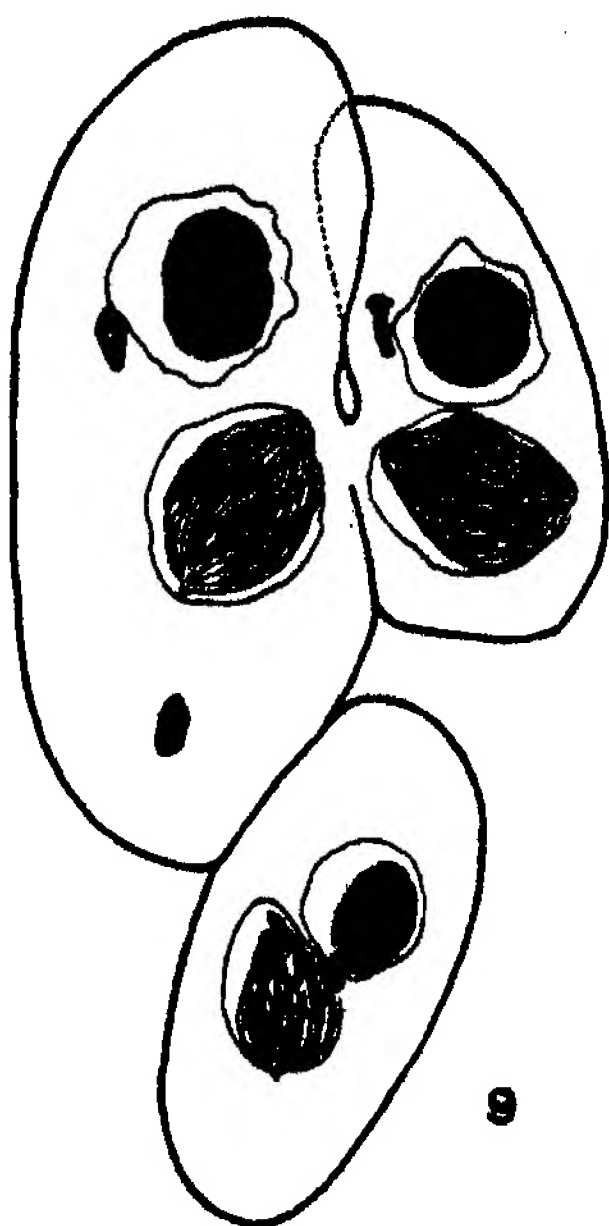
In a mixture containing a very large number of animals of each race several hundred "threes" may be found.<sup>4</sup> In the present study these "threes" were isolated some time before fixation and they were never mixed with the conjugating pairs during the process of fixation and subsequent staining. By isolating these conjugating "threes" from a single very large mixture and fixing a number of them every two hours during the entire period of conjugation (with subsequent staining) it was possible to trace the successive nuclear changes during conjugation of three animals and to determine the approximate time intervals between the different stages. In the present investigation this has been done. Other preparations were also made of "threes" which were isolated from other mixtures made at different dates which may be far apart. One or more sets of slides were made of the conjugants ("threes") from each mixture, each set containing animals which were fixed at one time. From the same mixture a number of "threes" may be fixed about six hours after onset



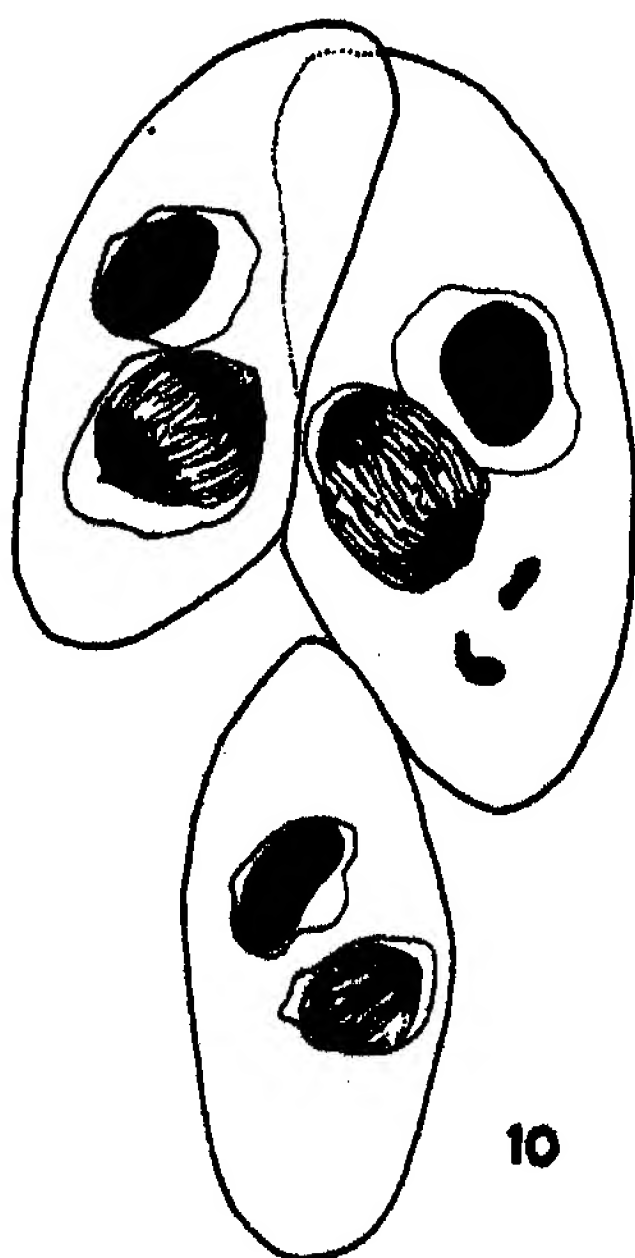
FIGURES 1-4  
For description see page 236.



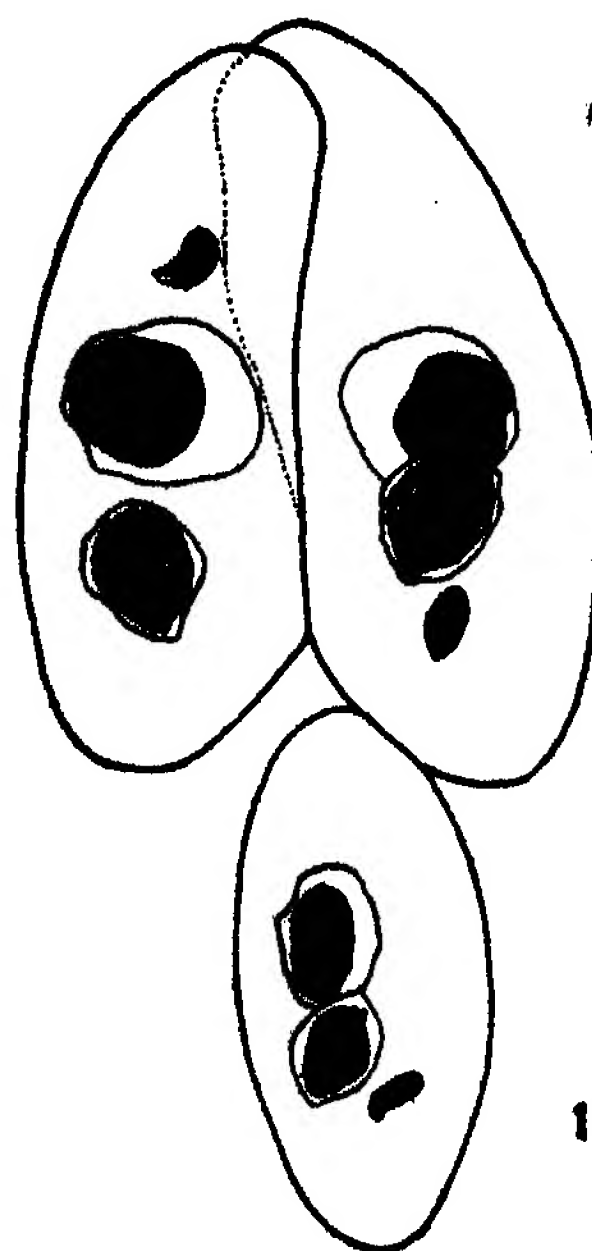
FIGURES 5-8  
For description see page 236.



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10



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FIGURES 9-11  
For description see pages 236-237.

## DESCRIPTION OF PLATES

## FIGURES 1-11

Conjugation of three animals in *Paramecium bursaria*. Schaudinn's fluid, Heidenhain's hematoxylin. All drawings  $\times 547$ .

Figure 1. *P. bursaria*. Race *McD*<sub>1</sub>, showing the relatively large, darkly stained micronucleus in the resting stage.

Figure 2. *P. bursaria*. Race *Fd*, showing the relatively small, lightly stained micronucleus in the resting stage.

Figure 3. Very early prophase of first pregamic division. The three conjugants can be identified by the size of the micronuclei and the quantity of chromatin they contain. Of the two anterior conjugants, the one on the left is *McD*<sub>1</sub>. Note its relatively large and darkly stained micronucleus. The anterior conjugant on the right is *Fd* which has a relatively small, lightly stained micronucleus. The third conjugant is also *Fd*, being attached to *McD*<sub>1</sub>. The micronucleus in each conjugant is considerably swollen. About 6 hours after onset of conjugation.

Figure 4. Early prophase of first pregamic division. Crescent-shaped micronuclei. Note the great difference between these two races in the quantity of chromatin contained in the micronucleus. Of the two anterior conjugants, the one on the left is *McD*<sub>1</sub>. Note the relatively large quantity of chromatin in the crescent-shaped micronucleus. The anterior conjugant on the right and the third conjugant belong to race *Fd*. Note the relatively small quantity of chromatin in their micronuclei. About 11 $\frac{1}{4}$  hours after onset of conjugation.

Figure 5. Late prophase of first pregamic division. Note the great difference between these two races in the number of chromosomes. Of the two anterior conjugants, the one on the left is *Fd* which contains apparently about eighty chromosomes. The anterior conjugant on the right is *McD*<sub>1</sub> which contains several hundred chromosomes. The third conjugant also belongs to race *Fd*. About 19 $\frac{1}{2}$  hours after onset of conjugation.

Figure 6. Anaphase of third pregamic division. Note the difference between the two races in the size of the micronucleus. The anterior conjugant on the left belongs to *Fd*, so does the third conjugant. They each have a relatively small micronucleus. The anterior conjugant on the right is *McD*<sub>1</sub> which has a relatively large micronucleus. In the cytoplasm of each of the three conjugants there are two degenerated micronuclei. About 29 $\frac{1}{2}$  hours after onset of conjugation.

Figure 7. Beginning of exchange of pronuclei. Of the two anterior conjugants, the one on the left is *Fd*. It contains two relatively small and lightly stained pronuclei. The anterior conjugant on the right is *McD*<sub>1</sub> which has two relatively large and darkly stained pronuclei. The third conjugant is *Fd*. In the third conjugant the migratory pronucleus moves to the vicinity of the mouth region even though the third conjugant does not make contact at the oral region with another conjugant. About 30 $\frac{1}{2}$  hours after onset of conjugation.

Figure 8. After the exchange of pronuclei. Exchange occurs only between the two anterior conjugants and never between the third conjugant and the conjugant to which it is attached. After the exchange of pronuclei each anterior conjugant possesses two pronuclei different in size and in staining capacity. The third conjugant which is *Fd* retains both small pronuclei. About 28 $\frac{1}{4}$  hours after onset of conjugation.

Figure 9. Formation of syncaryon. The syncaryon in one anterior conjugant resembles that of the other anterior conjugant. Autogamy in the third conjugant. The two pronuclei in the third conjugant are, in the present instance, not yet completely fused. Complete fusion of pronuclei in the third conjugant has been observed in some other "threes." About 30 $\frac{1}{2}$  hours after onset of conjugation.



Figure 10. Anaphase of first division of syncaryon. The two nuclei in the anterior conjugants are much larger and contain many more chromosomes than the nucleus in the third conjugant. About  $30\frac{1}{2}$  hours after onset of conjugation.

Figure 11. After the first division of syncaryon. One of the two daughter nuclei in each conjugant has degenerated. The remaining nucleus will divide twice before the conjugants separate. Note that the nucleus in the third conjugant is smaller than the nucleus in either anterior conjugant. About  $31\frac{1}{2}$  hours after onset of conjugation.

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of conjugation, another group of "threes" fixed about twelve hours after onset of conjugation, etc. The time intervals between the onset of conjugation and the time of fixation are known for all the material used in the present study.

There are several ways in which the three animals conjugate (Figs. A and B). Usually two of the three conjugants form an ordinary conjugating pair while the third conjugant attaches itself to the posterior part of one of these conjugants (Figs. B, 3-11). The third conjugant is usually attached to a conjugant belonging to a different race. The contact area between the third conjugant and the conjugant to which it is attached is usually small, sometimes very small.

Nuclear changes takes place in all of the three conjugants and at about the same speed as those in ordinary conjugating pairs of these races.<sup>5</sup> In many cases the nuclear changes are synchronous in all three animals, but often the nuclear events in the third conjugant lag behind. The single micronucleus in each conjugant undergoes three pregamic divisions (Figs. 3-6), resulting in the formation of two pronuclei (Fig. 7). These pregamic divisions are the same as those which occur in ordinary conjugating pairs. The first pregamic division is a very long process involving complicated changes. One of the two products of the first pregamic division degenerates, leaving one micronucleus which undergoes the second pregamic division. The second pregamic division requires a relatively very short time for its completion. One of the two products of the second pregamic division degenerates, leaving one micronucleus which undergoes the third pregamic division. This last pregamic division which also occurs rapidly gives rise to two pronuclei, one of which becomes migratory, the other stationary.

Exchange of pronuclei takes place only between the two anterior conjugants (Figs. 7, 8), never between the third conjugant and the conjugant to which it is attached. Such an exchange, if it had occurred between the third conjugant and the conjugant to which it is attached, would have easily been detected because of the difference in the size and staining capacity of the pronuclei between the two conjugants. It is particularly interesting to note that in the third conjugant there is also a differentiation of the two pronuclei into migratory and stationary pronuclei even though there is no exchange (Figs. 7, 8). The two pronuclei may exhibit differ-

ences in shape or in behavior or in both (Fig. 7). The migratory pronucleus in the third conjugant also moves to the vicinity of the mouth region just as if a conjugant were attached to that part of the body (Fig. 7). A bulge appears at this region of the body when the migratory pronucleus is against the cell membrane (Fig. 7). I have not observed a single case in which the migratory pronucleus in the third conjugant breaks through the cell membrane. Since there is no conjugant attached to the oral region, such a migratory pronucleus would probably be lost if it did break through. Instead, the migratory pronucleus of the third conjugant later fuses with the stationary pronucleus in the same conjugant to form a syncaryon (Figs. 8, 9). Autogamy thus occurs in the third conjugant. The syncaryon formed in the third conjugant (if it is an *Fd*) is much smaller than those in the other two (Figs. 9, 10). The syncaryon in each of the three conjugants undergoes three divisions (Figs. 10, 11) before the animals separate, as is the case in ordinary conjugating pairs.

Three facts concerning the behavior of pronuclei in conjugation of three animals seem to be significant: (1) As far as present observations go, the migratory pronucleus of the third conjugant never moves to the area of contact with the adjacent conjugant; (2) in the latter conjugant the migratory pronucleus, similarly, never moves to the area of contact with the third conjugant; (3) the migratory pronucleus of the third conjugant always moves to the vicinity of the mouth region as if a conjugant were attached to that region of the body. These facts seem to indicate that the path of the migratory pronucleus is predetermined.

The results of the present study show that:

(1) Contact between two conjugants at a small or even a very small region of the body is sufficient to initiate the usual nuclear changes accompanying conjugation in *Paramecium*.

(2) The path of the migratory pronucleus seems predetermined.

Details of the present investigation will be published elsewhere.

<sup>1</sup> Research Associate in Protozoölogy, School of Hygiene and Public Health, Johns Hopkins University.

<sup>2</sup> The word "conjugation" is here used to indicate the association of two or more individuals which results in the formation of gametic nuclei, irrespective of whether or not there are any interchanges of gametic nuclei between the joined individuals.

<sup>3</sup> I am greatly indebted to Prof. H. S. Jennings for the races of *P. bursaria* used in the present investigation. For genetical data on the two races of *P. bursaria*—*McD*, and *Fd*—see Jennings, *Genetics*, 24, 202-233 (1939).

<sup>4</sup> A set of three animals in conjugation is to be called a "three" in contradistinction to a pair.

<sup>5</sup> In a number of cases the nuclear changes in the third conjugant appear to be abnormal.

## POLYPLOIDY IN *PARAMECIUM BURSARIA*

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Communicated January 11, 1940

Although polyploidy is a very rare phenomenon among animals, it is commonly found in *Paramecium bursaria*. The present note is the first report of polyploidy in *Paramecium*.

*P. bursaria*—the green *Paramecium*—can be easily recognized because of the presence of symbiotic green algae in the cytoplasm. It has typically a single micronucleus of the "caudatum type." Jennings<sup>1</sup> distinguishes three groups of animals in *P. bursaria*. Members of one group will not conjugate with members of any other group. In each group there are a number of diverse mating types. In group I there are four mating types, in group II eight mating types and in group III four mating types. Animals belonging to the same mating type will not conjugate with each other but they will conjugate with animals of any other mating type within the same group.

Chromosomes were examined in ten races of *P. bursaria* belonging to group II collected from Maryland, North Carolina, Rhode Island and California.<sup>2</sup> It was found that in one race (*Fd*) the chromosome number is apparently about eighty, while in each of the other nine races (*McD*, *Gr6*, *Gr14*, *Fb*, *Fl*, *Wat16*, *HV1*, *Cal*, *JH8*) the chromosome number at the same stage is very much greater, running up to several hundred.

The size of the micronucleus in these ten races of *P. bursaria* is correlated with the number of chromosomes (figure 1). The race (*Fd*) which has a relatively small number of chromosomes has a small micronucleus which stains lightly and contains a small quantity of chromatin, whereas each of the nine polyploid races possesses a large, darkly staining micronucleus which contains a relatively large quantity of chromatin. The macronuclei in these races, on the other hand, are quite similar in size.

A probable explanation of this increased number of chromosomes is the occurrence of polyploidy, resulting from fusion of more than two pronuclei during conjugation. Evidence for this is the presence in the writer's material of a number of conjugants with three or four pronuclei. Cases have been observed in which these three or four pronuclei fused to form a syncaryon. In such conjugation the number of chromosomes is increased. The races with very large numbers of chromosomes possibly show the accumulated effects of several conjugations of this unusual type, which have occurred at various times in the past. The origin of this increased number of pronuclei in a conjugant probably lies either in (1) failure of one of the two products of the first or second pregamic division to degenerate, (2)

conjugation between a normal animal with one micronucleus and an animal with two micronuclei or (3) failure of the migratory pronucleus in one of the conjugants to migrate to the other conjugant. Hence in the one conjugant there are three pronuclei which appear to fuse (giving rise to polyploidy) and in the other conjugant there remains only the one stationary pronucleus.

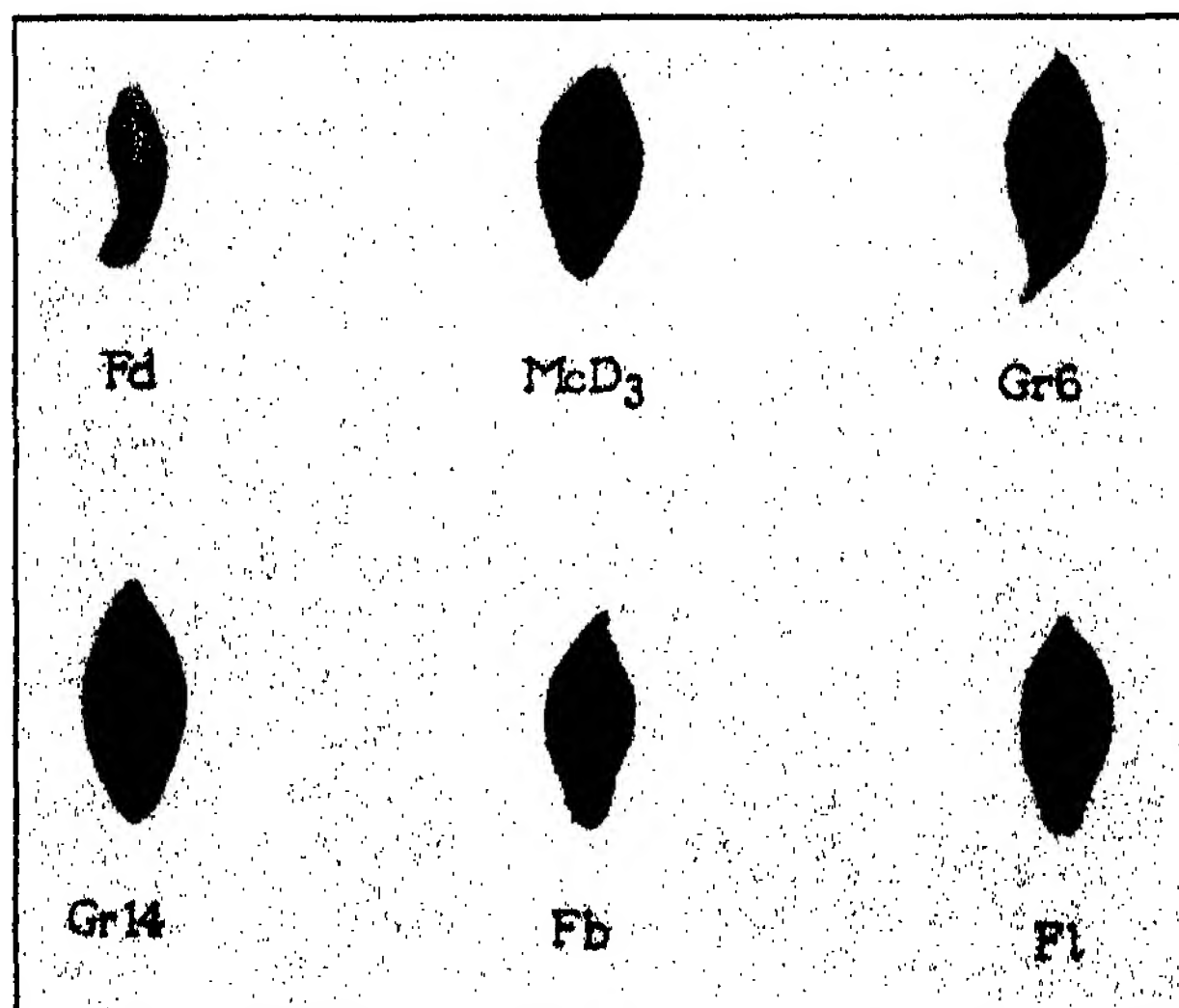


FIGURE 1

Micronuclei (resting stage) in six races of *P. bursaria*. The micronucleus in each race shows a characteristic size. Schaud. fl., Heid. haemat.  $\times 1640$ .

Polyploidy may also occur in *P. caudatum* judging from the report of Calkins and Cull<sup>3</sup> and the recent paper of Penn.<sup>4</sup> Penn claims that the diploid number in his race of *P. caudatum* is approximately 36 while Calkins and Cull found more than 150 chromosomes in their race of *P. caudatum*. If these two reports are accurate, they suggest polyploidy in *P. caudatum*.

<sup>1</sup> See Jennings, H. S., *Genetics*, 24, 202-233 (1939).

<sup>2</sup> I am greatly indebted to Prof. H. S. Jennings for these races of *P. bursaria*.

<sup>3</sup> Calkins, G. N., and Cull, S. W., *Arch. Protist.*, 10, 375-415 (1907).

<sup>4</sup> Penn, A. B. K., *Ibid.*, 89, 45-54 (1937).

## EVIDENCES OF EXCHANGE OF PRONUCLEI DURING CONJUGATION IN *PARAMECIUM BURSARIA*

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Communicated January 11, 1940

The problem of exchange of pronuclei during conjugation in *Paramecium* is of special importance from the standpoint of the genetics of these Protozoa. It has generally been assumed that such an exchange occurs during conjugation, but this has recently been questioned by Diller<sup>1</sup> and Wichterman.<sup>2</sup> Diller observes no exchange of pronuclei in *P. trichium*. Wichterman maintains just the opposite with respect to the same species. The latter also maintains that there is no exchange of pronuclei in *P. caudatum*.

Among the races of *P. bursaria* examined by the writer, some races show regular differences in the staining capacity and in the size of the micronucleus. In one particular race most of the animals have no micronuclei. These races were collected from Maryland and North Carolina and furnish most of the material used in the present study.<sup>3</sup> Preliminary studies on this problem of exchange of pronuclei were, however, made on races of *P. bursaria* which the writer collected from Virginia.

Under appropriate conditions, individuals belonging to different mating types (in the same group) will immediately agglutinate when mixed and soon form pairs. In such a mixture the progress of nuclear changes in all the pairs is quite uniform, especially in the earlier stages of conjugation. By fixing these conjugating pairs every hour (with subsequent staining) it was possible to trace with considerable accuracy the successive nuclear changes during conjugation and the time intervals between these different stages. Such successive nuclear changes and time intervals have now been worked out for *P. bursaria*. The time intervals between the onset of conjugation and the time of fixation are known for all the material used in the present investigation, excepting the material used in the preliminary study.

Conjugation in *P. bursaria* was obtained between animals with and without micronuclei, between animals with large and animals with small micronuclei and between animals with darkly staining and animals with lightly staining ("ghost") micronuclei.<sup>4</sup> During conjugation the micronucleus in each conjugant undergoes three pregamic divisions. After the first division, one of the two micronuclei degenerates. The remaining micronucleus undergoes the second pregamic division, giving rise to two micronuclei. One of the two daughter micronuclei degenerates, leaving only one micro-



nucleus which undergoes the third pregamic division. This last pregamic division gives rise to two pronuclei. Animals with large micronuclei produce large pronuclei; animals with small micronuclei produce small pronuclei; animals with darkly staining micronuclei produce darkly staining pronuclei; animals with "ghost" micronuclei produce "ghost" pronuclei. Amicronucleate conjugants produce no pronuclei. Because of these regularly distinguishing characteristics, it is possible to recognize the pronuclei belonging to different conjugants and to prove their exchange. These races of *P. bursaria* are thus ideal material for experimental studies of the exchange of pronuclei. The following description is a brief report of the results of these experimental studies.

1. *Conjugation between Animals with Large Micronuclei and Animals with Small Micronuclei.*—Race *McD*<sub>3</sub> has a characteristically large, darkly staining micronucleus containing a large quantity of chromatin. Race *Fd* has a characteristically small, relatively lightly staining micronucleus containing a small quantity of chromatin. Conjugation is readily obtained between these two races. During conjugation *McD*<sub>3</sub> produces two large, darkly staining pronuclei, whereas *Fd* produces two small, relatively lightly staining pronuclei. Thus the exchange of pronuclei is easily demonstrated. After the exchange, the two pronuclei in each conjugant (different in size, staining capacity and in quantity of chromatin) fuse to form a syncaryon.

2. *Conjugation between Animals with Darkly Staining Micronuclei and Animals with "Ghost" Micronuclei.*—Race *Gr14* has a characteristically large, darkly staining micronucleus. Race *S* contains some animals with a "ghost" micronucleus and other individuals without micronuclei. Conjugation is readily obtained between these two races. In conjugation between a *Gr14* individual and an *S* individual with a "ghost" micronucleus, the former produces two large, darkly staining pronuclei while the latter produces two "ghost" pronuclei. In such a mating an exchange of pronuclei occurs, after which each conjugant contains one darkly staining and one "ghost" pronucleus, respectively.

3. *Conjugation between Animals with Micronuclei and Animals without Micronuclei.*—Race *Gr14*, as stated in the previous paragraph, has a characteristically large, darkly staining micronucleus. Most of the individuals belonging to race *S* are without a micronucleus. Conjugation is readily obtained between races *Gr14* and *S* (without a micronucleus). In conjugation these amicronucleate animals produce no pronuclei. In *Gr14* two large, darkly staining pronuclei are formed in each conjugant. In most cases one of the two pronuclei in *Gr14* is transferred to the amicronucleate *S*.<sup>5</sup> After the transfer, each conjugant contains but a single pronucleus which later behaves like a syncaryon.

4. *Conjugation between Animals with Similar Micronuclei.*—In my preliminary study of the conjugation of *P. bursaria*, animals collected from



Virginia were used. All these animals have similar micronuclei. During conjugation the pronuclei in the two conjugants are alike. Under such circumstances evidence of exchange of pronuclei was less convincing although there were many pairs in which an exchange evidently occurred. The migratory pronucleus was usually slender, the stationary pronucleus relatively short and broad. In many cases, the migratory pronuclei were observed crossing from one conjugant to the other.

Exceptional cases found in the conjugation between animals having similar micronuclei further strengthen the evidence of exchange of pronuclei in *P. bursaria*. There is usually a simultaneous movement toward each other of the two migratory pronuclei in the two conjugants, but there are a few exceptional cases in which the movement is not simultaneous. One pronucleus completes its migration by the time the other begins. Such a behavior gives rise to pairs in which one conjugant appears to have three pronuclei whereas the other has one, clearly indicating that an exchange of pronuclei takes place during conjugation in *P. bursaria*.

<sup>1</sup> Diller, W. F., *Anat. Record* (supp.) 60, 92-93 (1934).

<sup>2</sup> Wichterman, R., *Biol. Bull.*, 73, 396-397 (1937); *Id.*, *Ibid.*, 75, 376-377 (1938).

<sup>3</sup> I am greatly indebted to Prof. H. S. Jennings for these races of *P. bursaria*.

<sup>4</sup> See Woodruff, L. L., *Quart. Jour. Micros. Sci.*, 74, 537-545 (1931).

<sup>5</sup> In some cases the two pronuclei in Gr14 remain in the same conjugant and fuse to form a syncaryon.

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## CONJUGATION IN *PARAMECIUM BURSARIA* BETWEEN ANIMALS WITH VERY DIFFERENT CHROMOSOME NUMBERS AND BETWEEN ANIMALS WITH AND WITHOUT MICRONUCLEI

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(1) *Conjugation between Animals with Very Different Chromosome Numbers.*—In *Paramecium bursaria* different races may have very different chromosome numbers. A study of ten races of *P. bursaria* shows that in one race (*Fd*) the chromosome number is apparently about eighty while in each of the other nine races the chromosome number is very much greater, running up to several hundred. A probable explanation of this increased number of chromosomes is the occurrence of polyploidy.<sup>1</sup>

Races of *P. bursaria* with very different chromosome numbers can conjugate with each other, for example, the two races—*McD*<sub>2</sub> and *Fd*.<sup>2</sup> These

two races belong to two different mating types and differ in the size of the micronucleus and in the quantity of chromatin contained in it. These differences are correlated with the difference in chromosome number. *McD*<sub>3</sub> is a polyploid race having several hundred chromosomes. It has a large, deeply staining micronucleus which contains a large quantity of chromatin. *Fd* has apparently about eighty chromosomes. This race has a small, lightly staining micronucleus which contains a small quantity of chromatin.

Under appropriate conditions, individuals belonging to these two races will immediately agglutinate when mixed and soon form pairs. In such a mixture the progress of nuclear changes is quite uniform in all the pairs; this is especially true in the earlier stages of conjugation. By isolating and fixing every hour a number of pairs from such a mixture (with subsequent staining) it is possible to trace with considerable accuracy the successive nuclear changes during conjugation and the time intervals between these different stages. In the present investigation this has been done. Many other preparations were also made of pairs which were isolated from other mixtures made at different dates which may be far apart. One or more sets of slides were made of the conjugants from each mixture, each set containing animals which were fixed at one time. From the same mixture a number of conjugants may be fixed about six hours after onset of conjugation, another group of conjugants fixed about eleven hours after onset of conjugation, etc. The time intervals between the onset of conjugation and the time of fixation are known for all the material used in the present study.

During conjugation between animals with very different chromosome numbers the nuclear changes in both conjugants are normal. The micronucleus in each conjugant undergoes three pregamic divisions. The first pregamic division is a very long process while the second and the third divisions require a very much shorter length of time for their completion. After the first pregamic division, one of the two micronuclei degenerates while the remaining one undergoes the second pregamic division, giving rise to two micronuclei. One of the two micronuclei from the second division degenerates, leaving only one micronucleus which undergoes the third pregamic division. This last pregamic division gives rise to two pronuclei. Throughout these stages it is clearly observed that the micronucleus in one conjugant (*McD*<sub>3</sub>) is much larger and contains a much greater quantity of chromatin than the micronucleus in the other conjugant (*Fd*). The two pronuclei in the *McD*<sub>3</sub> conjugant are also much larger and stain much more deeply than those in the *Fd* conjugant. At room temperature exchange of pronuclei usually occurs 28–31 hours after the onset of conjugation. After the exchange of pronuclei each conjugant contains one large, darkly staining pronucleus and one small, lightly staining pronucleus. These two pronu-

clei (different in size, staining capacity and in quantity of chromatin) in each conjugant fuse and form a syncaryon. The syncaryon in one conjugant appears to be the same as that of the other conjugant and they both divide three times before the conjugants separate. After the first division of the syncaryon, one of the nuclei degenerates, and the other divides twice, giving rise to four nuclei. The nuclei resulting from the divisions of the syncaryon are alike in both conjugants. Thus before conjugation the two conjugants are different in the size of the micronuclei and in the number of chromosomes but after conjugation they are alike.

(2) *Conjugation between Animals with and without Micronuclei.*—In *P. bursaria* animals with and without micronuclei can conjugate with each other, for example, the two races—*Gr14* and *S*. They belong to two different mating types. Race *Gr14* has a characteristically large, darkly staining micronucleus. In race *S* most of the animals do not have a micronucleus, while some have a “ghost” micronucleus which does not stain with haematoxylin. Conjugation is readily obtained between these two races.<sup>3</sup> A *Gr14* individual can conjugate either with an *S* individual without a micronucleus or an *S* individual with a “ghost” micronucleus.

During conjugation between *Gr14* and the amiconucleate *S*, the micronucleus in *Gr14* shows a normal behavior. It undergoes three pregamic divisions, resulting in the formation of two pronuclei. These pregamic divisions are similar to those found in conjugation between races *McD<sub>3</sub>* and *Fd*. The amiconucleate conjugant produces no pronuclei. In most of the pairs one of the two pronuclei in the *Gr14* migrates to the amiconucleate conjugant. After this migration each conjugant possesses a single pronucleus (“hemicaryon”).<sup>4</sup> Since *Gr14* is a polyploid race, each “hemicaryon” probably still contains several sets of chromosomes. The “hemicaryon” in each conjugant behaves like a syncaryon. It undergoes three divisions before the conjugants separate. After the first division, one of the nuclei degenerates, leaving one nucleus which undergoes two divisions, giving rise to four nuclei. The nuclei resulting from these divisions of the “hemicaryon” in one conjugant appear to be the same as those of the other conjugant and they all seem to contain the same number of chromosomes. Thus before conjugation the two conjugants are different in the nuclear apparatus but after conjugation they are alike.<sup>5</sup>

The account given above of the transfer of one pronucleus from the *Gr14* conjugant to the amiconucleate conjugant is true for most of the conjugating pairs. In a small number of exceptional cases, no such transfer takes place. The two pronuclei in *Gr14* remain in the same conjugant and fuse to form a syncaryon. A syncaryon resulting from such autogamy behaves like those formed after an exchange of pronuclei. After the first division of the syncaryon, one of the two products degenerates and the remaining nucleus divides twice, giving rise to four nuclei. The syncaryon

and its products of divisions are larger and contain many more chromosomes than the "hemicaryon" and its products of divisions. This is to be expected since there is no reduction of chromosome number in autogamy, whereas in the case in which the transfer of pronucleus takes place the chromosome number is probably halved.

The significance of conjugation between animals with very different chromosome numbers and between animals with and without micronuclei seems to be twofold:

(1) There is considerable flexibility in conjugation of *Paramecium*. Animals with a great number of chromosomes can conjugate with animals with relatively few chromosomes. Animals with the usual micronucleus can conjugate with animals without a micronucleus.

(2) One of the results of conjugation in *Paramecium* is the elimination of the great diversities in the nuclear apparatus and great difference in chromosome number that may exist between the two conjugants.

<sup>1</sup> See Chen, T. T., *Proc. Nat. Acad. Sci.*, 26, 239-240 (1940).

<sup>2</sup> I am greatly indebted to Prof. H. S. Jennings for the races of *P. bursaria* used in the present investigation.

<sup>3</sup> The technique used in the present study of conjugation between *Gr14* and *S* is essentially the same as that used for the study of conjugation between *McD<sub>3</sub>* and *Fd*.

<sup>4</sup> As soon as this pronucleus in each conjugant starts to divide it should no longer be called a "pronucleus." A new term "hemicaryon" is hereby used in contradistinction to syncaryon. A syncaryon is made up of two (or more) pronuclei, whereas a "hemicaryon" is composed of only one pronucleus.

<sup>5</sup> Diller reported in *P. aurelia* cases of conjugation in which one member of the pair was normal while the other member lacked micronuclei entirely. He did not describe the nuclear changes in the normal conjugant nor the result of such conjugation. See Diller, W. F., *Jour. Morph.*, 59, 11-51 (1936).

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## NOVEL TYPES OF NERVE REFLEXES

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The idea of the nerve reflex has since the time of Descartes played a rôle of first importance in neurophysiology (Fearing, 1930). Like the much later concept of the neurone, as formulated by Waldeyer in 1891, it has afforded a basis of first significance for the analysis of nervous activities. Neurones have proved to be of many kinds, but the nerve reflex has remained almost true to type. Sherrington has called it the unit of functional nervous integration. Pavlov divided reflexes into the well-known

non-conditioned and conditioned, but aside from this grouping almost no step has been taken to distinguish different kinds. The discovery of hormones and particularly of those hormones that are especially concerned with nervous activity, the chemical activators or neurohumors, has brought a new element into the field of the reflex.

As ordinarily understood, a nerve reflex is represented by a series of nerve impulses that pass over an afferent pathway leading from some bodily receptor to a central nervous organ whence it is reflected into an efferent pathway by which it reaches a peripheral effector usually a muscle or a gland. In such an instance both pathways are strictly nervous and a pure nerve reflex results. But with the discovery that neurohumors may replace nerve impulses new combinations appeared. This is well seen in the activity of the melanophores in such an animal as the catfish *Ameiurus* (Parker, 1934, 1935, 1936).

This fish darkens when it is placed in a black-walled receptacle brightly illuminated. Under such circumstances the pigment granules which in its myriads of integumentary color cells form in each cell a minute spherical mass spread throughout the branched body of the cell and thus come to cover a relatively large area. This dispersion of pigment is brought about in two ways, by the action of dispersing nerve-fibres and by the presence of intermedin, a substance secreted by the intermediate lobe of the pituitary gland in the base of the fish's brain.

When light from a black, illuminated surface falls upon the retina of a catfish, nerve impulses are generated which pass over the optic nerve to the central nervous organs. Here these impulses are transferred to nerves that leave the central organs and pass to the melanophores which are by the action of these nerves excited to disperse their pigment. This operation represents in all respects the well-known type of nerve reflex. The nerve connections from the eye to the central organs form the afferent path and those from the central organs to the melanophores the efferent path.

The mechanism of color change accomplished by intermedin is of a different character. The light which falls upon the skin of the fish excites certain photoreceptors by which nerve impulses are induced which then pass over afferent nerve tracts to the pituitary gland. The innervation of this gland in connection with the color changes of the frog has been recently worked out by Geiringer (1938). As a result of the nervous excitation of this gland in the catfish, intermedin is liberated and is conducted by means of the blood and lymph to the melanophores of this fish. These color cells are thereby excited to disperse their pigment. Thus, a result is obtained which in all essential respects is like that of the purely nervous reflex, though by somewhat different means. In the first type, both afferent and efferent arms of the reflex are nervous, but in the second, only the afferent one is nervous, the efferent arm being humoral. This humoral



part is represented by the flow of intermedin in the circulatory fluids of the fish from the pituitary gland to the responding melanophores.

The type of reflex whose afferent arm is nervous but whose efferent one is humoral, must have been in the minds of many students of the physiology of vertebrate chromatophores, but no one, so far as I am aware, has ever called attention to it. It is widely exemplified in the chromatophoral systems of the great majority of chromatic vertebrates. In consequence of the different kinds of activities on its two arms, it may well be designated a neurohumoral reflex as contrasted with the purely neural reflex long since known.

If reflexes exist in which the afferent path is nervous and the efferent one humoral, it is not impossible that others may occur in which the reverse is true. Such seems to be the case in the respiratory reflexes of the higher vertebrates. Here the afferent path is represented by the course of the blood from the tissues of the body to the respiratory center in the medulla oblongata whereby the exciting hormone, be it carbon dioxide or other metabolite, is carried from its region of origin to the respiratory center in the nervous system. The efferent path, on the other hand, consists of the nerve connections leading from the respiratory center to the muscles of respiration. Thus, in this instance, the afferent path is humoral and the efferent nervous, the reverse of those in the example taken from the melanophores.

If the second type of melanophore reflex in consequence of the sequence of its steps may be called a neurohumoral one, the respiratory reflex for a similar reason may be designated a humeroneural reflex. Such a system may be pursued one step further. There are many instances in the bodies in the higher animals where what might be assumed to be reflex arms are both humoral. Such cases are to be met with in the activating mechanism of the pancreas and among the interacting sex hormones, but it is doubtful if any of these examples may legitimately be called reflexes because of the complete absence of a nervous factor. The combinations presented by nerve and humoral elements, as exemplified in the catfish, seem to justify at most three types of true reflexes, the relatively simple and long recognized purely nervous type and the two combination types neurohumoral and humeroneural. A brief note on this subject has already appeared in *Science* (Parker, 1940).

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## AGGLUTINATION OF SEA-URCHIN EGGS BY MEANS OF A SUBSTANCE EXTRACTED FROM THE EGGS

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F. R. Lillie<sup>1</sup> showed that filtrates from a suspension of ripe eggs of the sea-urchin, *Arbacia*, will agglutinate the sperm of the same species. This species-specific agglutination of sperm by a filtrate of an egg suspension occurs in a number of forms of animals (see Just,<sup>2</sup> Tyler<sup>3</sup>). The agglutinin is, according to the latest evidence<sup>4,5</sup>, the jelly coat of the egg or a component of it. Lillie<sup>6</sup> assumed that in addition to the agglutinin (fertilizin) there was another substance in the egg which he termed anti-fertilizin and which had the capacity of neutralizing the sperm-agglutinin. I have been able to extract such a substance from eggs of the sea-urchin, and have found that not only does the extract neutralize the sperm-agglutinin but also that it can cause the intact eggs to agglutinate. The substance in the extract that neutralizes the sperm-agglutinin may be termed anti-sperm-agglutinin, rather than anti-fertilizin since we do not as yet know what relation it has to the fertilization process. This substance is, according to the present evidence, identical with the substance in the extract that causes agglutination of the eggs, but it may be preferable to refer to the latter as the egg-agglutinin. It is obtained simply by extracting the eggs after removal of the jelly coat.

*Extraction of Jellyless Eggs.*—The jelly layer (Fig. 1c) of eggs of the sea-urchin *Strongylocentrotus purpuratus* can be readily removed (Fig. 1d) by acidifying the egg suspension to about pH 3.5. When a suspension of washed, jellyless eggs in ordinary sea water is frozen at  $-78^{\circ}\text{C}$ . and allowed to thaw at room temperature the eggs break up and there is obtained a yellowish, colloidal solution along with a coagulum of insoluble egg material.

*Inactivation of Sperm-Agglutinin by Egg Extract.*—When the solution (extract of jellyless eggs) is added in the proper amount to a sperm-agglutinin solution (filtrate of egg suspension), the latter completely loses its

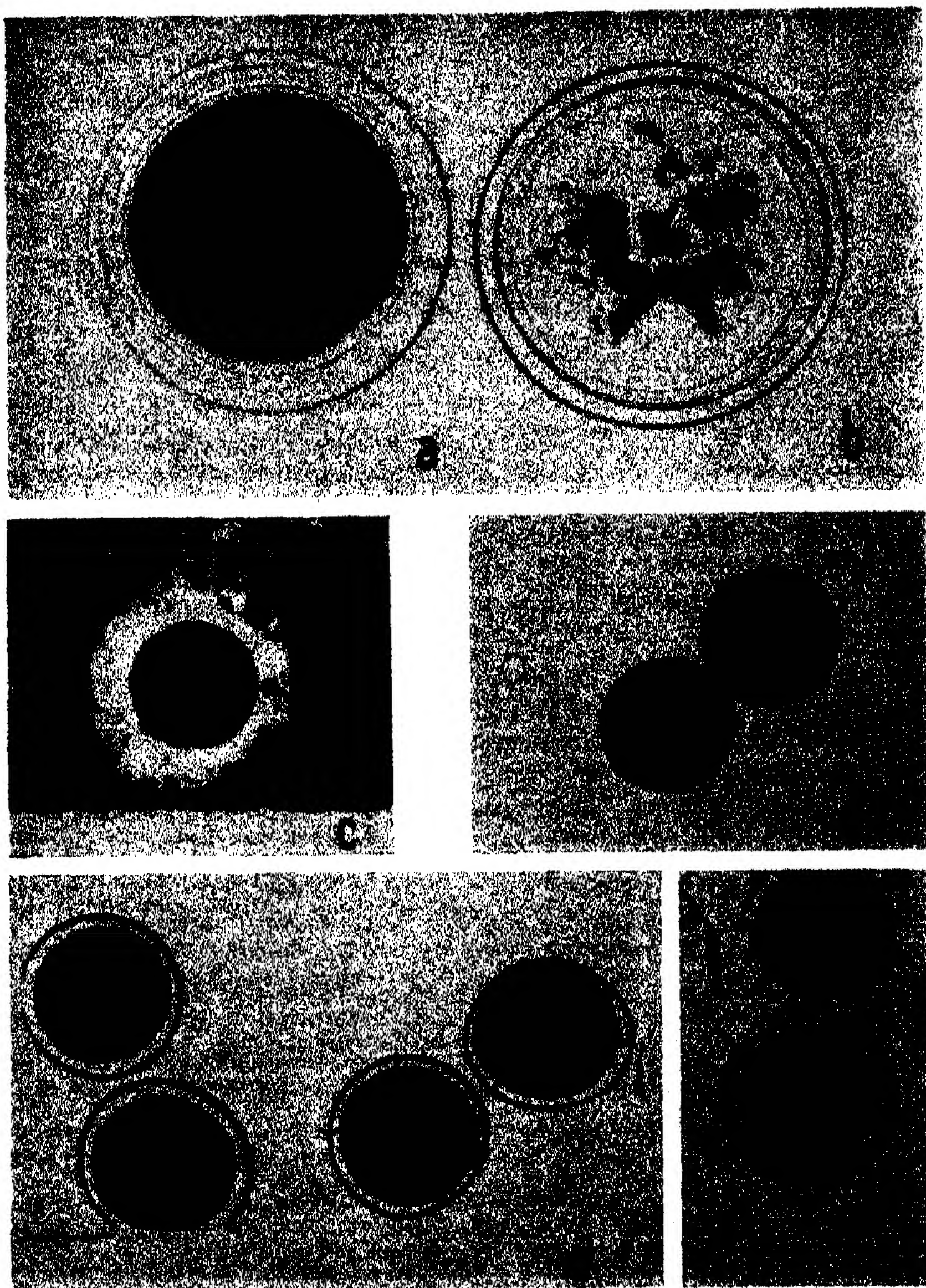


FIGURE 1

*a* and *b*. Photograph of egg suspensions in Syracuse dishes; 7/10 natural size; *a*, suspension in ordinary sea water; *b*, 15 minutes after the addition of extract of jelly-less eggs to a suspension of approximately the same concentration as in *a*, showing agglutination of the eggs.

capacity to agglutinate sperm. With concentrated solutions a precipitate forms on mixing the egg extract with the egg filtrate. In other words, a typical precipitin reaction is obtained on mixing the two solutions.

*Agglutination of Intact Eggs by Egg Extract.*—When the extract of jellyless eggs is added to a suspension of intact eggs it causes them to agglutinate (Fig. 1a and b). With the proper concentrations pH, etc., practically all of the eggs form one large clump. The microscopic picture shows that the substance in the extract reacts with the material of the jelly layer of the egg. There is formed on the surface of the jelly layer a precipitate that gives this layer a sharp outline (Fig. 1e). The precipitate has the appearance of a membrane and may be termed a precipitation membrane. In the agglutinates of intact eggs the precipitation membrane is shared between adjacent eggs and is thinner in the region of contact than elsewhere. Isolated eggs in strong egg extract are surrounded by a complete precipitation membrane. This effect resembles very much the action of anti-serum on the capsules of various encapsulated bacteria such as the pneumococci. But while the capsule of such cells is considered by bacteriologists to swell (Neufeld's Quellung) on forming the precipitation membrane, the jelly layer of the sea-urchin egg shrinks appreciably. The formation of a precipitation membrane is a more reliable and rapid test of the active substance than is the actual clumping of the eggs since the latter depends more on having proper concentrations, pH, etc.

*Inactivation of Egg-Agglutinin by Sperm-Agglutinin.*—This is the same experiment reported two sections above except that the egg-agglutinating activity of the mixture of egg-agglutinin (extract of jellyless eggs) and sperm-agglutinin (filtrate of egg suspension) is tested. The egg-agglutinin is found to be completely inactivated when a sufficient amount of sperm-agglutinin is added.

*Fertilization of Eggs Treated with Egg Extract.*—Eggs with a complete precipitation membrane cannot be fertilized. However, if the membrane is removed or torn even only partially, the eggs can be fertilized. In the latter case a normal fertilization membrane forms within the incomplete precipitation membrane (Fig. 1f).

*Properties and Relationship of the Active Agents in the Egg Extract.*—The identity of the anti-sperm-agglutinin and the egg-agglutinin was surmised on the basis of the evidence that the material of the jelly-layer of the egg

FIGURE 1 (Continued)

c to f. Photomicrographs of eggs,  $\times 200$ ; c, an unfertilized egg in a suspension of Chinese india ink showing clear jelly layer surrounding the eggs; d, two eggs after removal of the jelly layer, the absence of which is shown by the proximity of the eggs; e, four eggs at 10 minutes after the addition of egg extract, showing precipitation membrane on surface of the jelly; f, a fertilized egg showing fertilization membrane formed inside of a nearly complete precipitation membrane.



is the sperm agglutinin and that the egg extract reacts visibly with that material. The evidence is strengthened by the fact that both are inactivated similarly by heat, pH and proteolytic enzymes. Also both are non-dialyzable, are precipitated by saturated  $(\text{NH}_4)_2\text{SO}_4$  and by sperm-agglutinin. These properties are utilized in concentrating and attempting to purify the active agent.

Preparations obtained after removal of stromata, dialysis and precipitation with  $(\text{NH}_4)_2\text{SO}_4$  give the common color tests for proteins (xanthoproteic, Millon's and biuret). Solutions of crystallized trypsin and chymotrypsin inactivate the egg-agglutinin (and anti-sperm-agglutinin). This latter test is complicated by the fact that these enzymes also act on the sperm-agglutinin (egg jelly) but by proper controls they can be shown to act separately on the egg-agglutinin. The evidence, then, points to the substance being of protein nature.

*Anti-Sperm-Agglutinins and Egg-Agglutinins from Sperm Extracts.*—From the spermatozoa of the sea-urchin and the keyhole limpet there have also been extracted recently specific anti-sperm-agglutinins (Frank,<sup>6</sup> Tyler,<sup>7</sup> Southwick<sup>8</sup>). Frank<sup>6</sup> showed that the extract of sea-urchin sperm also has the property of causing agglutination of the eggs. I have been able to confirm this on extracting the sperm by freezing and thawing instead of heating as Frank had done. While Frank hesitates to conclude that the anti-sperm-agglutinin from sperm and the egg-agglutinin from sperm are identical, his and my own evidence point very strongly to the view that they are. Frank failed to obtain with his extracts any protein tests nor was he able to salt it out with  $(\text{NH}_4)_2\text{SO}_4$ . My extracts, on the other hand, give positive protein tests and are precipitable by  $(\text{NH}_4)_2\text{SO}_4$ . This may be due to the greater efficiency of extraction by freezing and thawing than by heating, since extracts that I have prepared by heating are of lower titer than those obtained by freezing and thawing.

*Relation of Active Agent from Sperm to That from Egg Extract.*—The egg-agglutinin (and anti-sperm-agglutinin) obtained from sperm also resembles the egg-agglutinin (and anti-sperm-agglutinin) obtained from jellyless eggs in regard to its non-dialyzability and its inactivation by heat, pH and proteolytic enzymes. It is for further work to decide if the two are identical. In the sperm the substance must be present on the surface since it is quite evidently the substance that reacts with the sperm-agglutinin obtained from the egg jelly. In the eggs the substance is present below the jelly-layer.

*Specificity.*—All of the various substances that have been described here are species specific. Cross reactions are in some instances obtained between the closely related sea-urchins *S. purpuratus* and *S. franciscanus*. These are usually weaker than the homologous reactions. More distantly related Echinoderms show no cross reactions at all.

## SOME DEDUCTIONS OF GENERAL SEROLOGICAL SIGNIFICANCE

1. *Auto-Agglutination*.—The present finding that there is below the surface of a cell a substance capable of giving the familiar serological reactions (agglutination, precipitation) with the surface materials of the cell has a bearing on some general problems. One of these is the cause of the phenomenon of auto-agglutination (acid-, cold-agglutination and spontaneous agglutination in general). This phenomenon, it appears, can now be explained in the following manner. Surface and sub-surface substances are assumed to form a dissociable compound in the region in which they adjoin. Agents that dissolve the surface substance would then favor the dissociation of this compound. Within a wide range of conditions this dissociation will be incomplete so that the surface will then be a mosaic of original surface substance and sub-surface substance. The cells are then capable of uniting in large numbers, the surface substance of one combining with the exposed sub-surface substance of another. The same result would follow if it were assumed that complete dissociation occurred in some of the cells and no dissociation in the others.

This interpretation of auto-agglutination presupposes the same mechanism for agglutination as that assumed in the lattice theory of Marrack<sup>9</sup> and Heidelberger;<sup>10</sup> namely, the union of multivalent complementary substances.

I have recently<sup>3</sup> offered this interpretation for an auto-agglutination phenomenon in spermatozoa. A critical test is the ability to agglutinate cells by means of an extract from below the surface layer of the cells. The present findings show that this can be done with eggs. It is also possible to produce an auto- (acid-) agglutination of the eggs in a very simple manner. If the pH of the sea water is lowered to a point where the jelly is almost completely gone the eggs will agglutinate in large clumps. It is, of course, difficult to determine whether there are patches of very thin films of the jelly layer present under these conditions, but that appears to me to be the interpretation. The effect may be produced also by using chymotrypsin. This enzyme dissolves the jelly layer and when that layer is almost (?) completely gone the eggs agglutinate. As the chymotrypsin continues to act the clumps break up again, presumably due to the complete removal of the surface material.

The auto-agglutination of egg cells substantiates, then, the view that the effect is due to the exposure of sub-surface material capable of combining with surface material on other cells of the same suspension. Whether or not this interpretation will hold for all instances of auto-agglutination that have been described remains for future investigations to decide.

2. *The Terms Antigens and Antibodies*.—Serologists employ the terms normal or natural antibodies to designate substances that are present in untreated animals and that produce effects similar to those of antibodies

obtained by injection of antigens. Thus the naturally occurring agglutinins, lysins, etc., for blood cells and bacteria are called natural antibodies. The substance with which a natural antibody reacts is termed an antigen (agglutinogen, precipitinogen, etc.). This, however, conflicts with the complete definition of an antigen since there is no evidence that the formation of a natural antibody is incited by the corresponding antigen. Nevertheless there seem to be cogent reasons for employing the term.

When one attempts to use these terms in connection with the substances obtained from eggs and spermatozoa further difficulties are encountered. These difficulties do not reflect on the character of the reactions exhibited, since they are typical serological reactions, but rather on the terminology itself.

The substance on the surface of the egg that causes agglutination of the sperm may be termed a natural agglutinin. Then the reacting substance on the sperm would according to serological usage be termed an agglutinogen. However, the agglutinogen can be extracted from the sperm and when added to eggs causes them to agglutinate. It thereupon must be called an agglutinin.

The substance below the surface of the egg which, when added to intact eggs, causes them to agglutinate may be termed a natural agglutinin. Then the surface substance with which it reacts becomes an agglutinogen.

It is evident that if these terms are to be employed when dealing with the naturally occurring substances, they must be used in their functional sense. In the description of the experiments with eggs and sperm it was necessary to employ such cumbersome terms as egg-agglutinin or anti-sperm agglutinin from eggs, etc. This avoids the difficulty of designating a substance as an antigen (agglutinogen) at one time and an agglutinin at another time.

That this difficulty in terminology is not confined to the naturally occurring substance is manifest when one considers the production of antibodies to antibodies. Several instances showing the production of anti-antibodies have been reported (see Marrack,<sup>9</sup> p. 54). The antibody-inducing antibody must then be termed an antigen. Again it is evident that these terms must be employed in a functional sense and not as designating special classes of chemical entities.

These specifically reacting substances may be termed complementary substances (complementary proteins, etc.) but any further characterization must await their isolation in pure form.

3. *Possibility of Direct Protective Action of Bacterial Extract against Intact Bacteria.*—These experiments with egg cells have shown that there is below the surface of the cell a substance capable of acting in the manner of an antibody (agglutinin, precipitin) with the surface material. This has certain implications that should be of considerable interest in the



medical aspects of immunology. If, in analogy to the eggs, a pathogenic bacterium were to have beneath its surface a substance capable of agglutinating the cells, then, on the basis of the current views concerning the immunological significance of specific agglutinins (see Cannon<sup>11</sup>), one should expect this substance to afford protection to an animal infected with the same strain of organism. In other words the bacteria would contain within themselves the seeds of their own destruction.

Some experiments that have been performed with bacteriophage are consistent with this view. Levine and Frisch<sup>12</sup> and Gough and Burnet<sup>13</sup> showed that phage can be inactivated by a polysaccharide-like substance extracted from the susceptible bacteria. The substance is presumably the coat of the organism or located in it. Within the cell there is evidently material that serves as phage-precursor. It is reasonable to suppose that the precursor is a fully formed protein and that the phage is not synthesized from simpler compounds but is produced in a manner analogous to the formation of an enzyme from its precursor (see Northrop<sup>14</sup>). The situation (at least just before liberation of the phage) is then similar to that demonstrated here in the case of the sea-urchin egg; namely, a sub-surface substance capable of combining in a serological manner with surface substance.

The views expressed here should be relatively easy to test. All that is necessary is to remove the surface material from a cell and to determine whether by appropriate extraction of the remainder a substance can be obtained that will react with the surface material. Depending on the conditions of the test the reaction should give precipitation, agglutination, lysis or opsonification. In other words the extract would be expected to have the same properties as an immune serum. An attempt to test this on pneumococci is now in progress.

The results of numerous experiments in the literature on immunology may find their interpretation on the basis of the results presented here. For example, the differences in effectiveness of various kinds of vaccines may be dependent upon the relative amounts of surface and sub-surface (and deeper) substances present when different methods of preparation are employed. Autolysis or cytolysis of a whole cell would allow a general interaction of surface and sub-surface and deeper substances to take place. A precipitate would form and the substances that remained in solution would be the ones present in excess. Since the antibodies that are effective in producing immunity are evidently those that act on the surface of the cell, it would appear that it is necessary to have the proper antigen present in the vaccine or extract. Immunologists are, of course, fully aware of the importance of isolating the various antigenic components of cells. The new points submitted here are that the various components of the cell are capable of reacting with one another when the cell is destroyed and are

chemically combined in the intact cell in the regions where they adjoin one another.

*Summary.*—Sea-urchin eggs can be agglutinated by means of an extract obtained from eggs deprived of their jelly coat. The extract also inactivates the sperm-agglutinin which is the jelly coat of the egg or a component of it. Similarity of properties point to the identity of egg-agglutinin and anti-sperm-agglutinin obtained from eggs. These agents also appear to be similar to the egg-agglutinin and anti-sperm agglutinin obtained from sperm. Various tests indicate the protein nature of the egg-agglutinin (and anti-sperm-agglutinin). The egg agglutinin is specific in its action.

The view is proposed that all cells are composed of alternate layers of substances that are capable of reacting with one another in a serological manner. The bearing of this on some general serological and immunological questions is discussed. One deduction of considerable practical interest is that it should be possible to extract from a pathogenic bacterium a substance that would give direct protective action against the intact bacterium.

I am indebted to Professors T. H. Morgan and Linus Pauling for having read the manuscript.

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<sup>12</sup> P. Levine and A. W. Frisch, *Proc. Soc. Exp. Biol. Med.*, **31**, 46 (1933).

<sup>13</sup> G. A. C. Gough and F. M. Burnet, *Jour. Path. Bact.*, **38**, 301 (1934).

<sup>14</sup> J. H. Northrop, *Crystalline Enzymes*, Columbia University Press, New York (1939).

# DIURNAL VARIATION OF INTERMEDIN IN THE BLOOD OF THE ALBINO RAT

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It has been found by Jores (1937)<sup>1</sup> that there is a diurnal fluctuation in the concentration of intermedin in the blood of man. The following experiments were therefore designed to test the possibility of the existence of such a cycle in the albino rat.

*Methods.*—A supply of mature, normal albino rats, of both sexes, was kept under normal laboratory conditions. Blood samples were obtained by heart puncture and the samples prepared for assay in the following manner, an adaptation of that of Jores (1933).<sup>2</sup> In approximately one-half of the cases, 0.3 cc. of blood was withdrawn and added to 5.0 cc. of 70% alcohol. In the other cases, 0.6 cc. of blood was taken, and added to 10.0 cc. of 70% alcohol, in a small centrifuge tube. After shaking, the laked hemoglobin was removed by centrifugation, and the supernatant solution evaporated to dryness in a blast of warm air. The residue was taken up in an amount of water such that each cubic centimeter of solution contained the equivalent of 0.12 cc. of blood. This aqueous solution was made alkaline (about pH 11) with 0.1 *N* NaOH, and heated for 10 minutes in a boiling water bath. After cooling, the samples were neutralized with 0.1 *N* HCl, and diluted to replace the loss due to evaporation. 0.5 cc. of this solution was injected into pale frogs, and the duration of the resulting melanophore expansion determined. This was measured as the length of time in minutes between the time of injection and the time at which the frogs returned to the pale state.

*Results.*—The results obtained by assaying the intermedin in the blood of rats at different times of day and night are shown in tabular form in table 1, and graphically in figure 1. It can be seen that intermedin is al-

TABLE 1

CYCLICAL VARIATIONS IN THE INTERMEDIN CONTENT OF RAT BLOOD

I TIME OF DAY	II NO. OF RATS TESTED	III MEAN DURATION OF RESPONSE	IV S. D. OF DURATION OF RESPONSE
12 mdnt.	10	231.0 mins.	82.7 mins. = 36%
3 A. M.	8	215.6	105.5 mins. = 49%
6 A. M.	8	189.4	69.5 mins. = 37%
9 A. M.	4	167.5	24.8 mins. = 37%
12 noon	4	148.8	15.6 mins. = 32%
3 P. M.	11	198.0	32.6 mins. = 33%
6 P. M.	8	137.5	33.4 mins. = 24%
9 P. M.	6	128.3	40.7 mins. = 32%

ways present in the blood of the rat, but that more is present during the night than during the day. Moreover, the concentration of intermedin varies cyclically, in a fashion roughly similar to the diurnal cycle in spontaneous activity (Slonaker, 1935).<sup>3</sup>

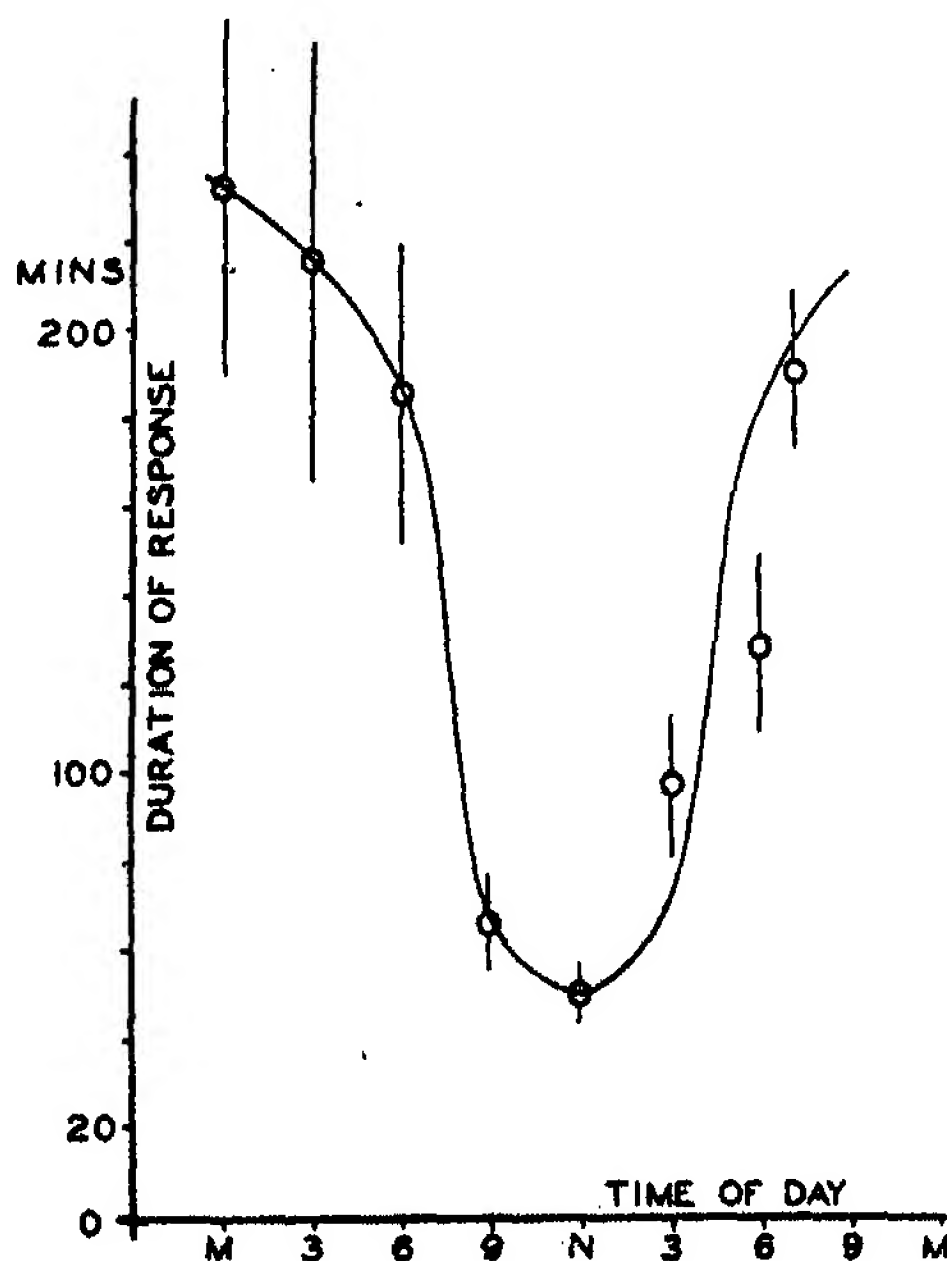


FIGURE 1

## EXPLANATION OF CURVE

This curve shows the relationship between the duration of melanophore expansion in minutes (ordinate) and the time at which blood intermedin samples were taken (abscissa). The circles represent average values from table 1, with their standard deviations expressed as the vertical lines at each point.

M = midnight

N = noon

The author wishes to express his thanks to Dr. A. A. Abramowitz for his generous and helpful advice.

<sup>1</sup> Jores, A., *Zeits. Exper. Med.* 87, 266 (1937).

<sup>2</sup> Jores, A., *Tabulae Biologicae*, 14, 77 (1933).

<sup>3</sup> Slonaker, J. R., *Am. Jour. Physiol.*, 73, 485 (1925).

## THE ABSOLUTE MAGNITUDES OF STARS OF HIGH LUMINOSITY

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Available estimates of the distances of stars of high luminosity do not establish a satisfactory scale of absolute magnitudes. The theory of galactic rotation, however, affords a geometric extrapolation of known distances, by the use of the radial velocity as a measure of distance. If we assume that higher-order terms in the galactic rotation may be neglected the radial velocity of a star,  $\rho$ , after correction for the solar motion, is

$$\rho = rA \sin 2(l - l_0) \cos^2 b + \delta\rho. \quad (1)$$

$\delta\rho$  is the component of the peculiar motion in the line of sight. The rotational velocity gradient,  $A$ , may be assumed known from near-by stars for which the distances are determined by secular parallaxes. The mean distance of a group of stars is

$$\bar{r} = \frac{1}{A} \left( \frac{\rho}{\sin 2(l - l_0) \cos^2 b} \right). \quad (2)$$

The average deviation of a distance will be

$$|\overline{\delta r}| = \frac{|\overline{\delta\rho}|}{A} \left( \frac{1}{\sin 2(l - l_0) \cos^2 b} \right), \quad (3)$$

and of an absolute magnitude,

$$|\overline{\delta M}| = \frac{2.17 |\overline{\delta\rho}|}{rA} \left( \frac{1}{\sin 2(l - l_0) \cos^2 b} \right). \quad (4)$$

We may estimate the *mean error* of the mean absolute magnitude of  $n$  stars to be

$$\text{m. e.} = \frac{4.4 |\overline{\delta\rho}|}{rA \sqrt{n - 1}}, \quad (5)$$

when only those stars are selected for which  $\sin 2(l - l_0) \cos^2 b \geq 0.5$ . The value of the mean peculiar velocity of the supergiants is not established. Using known  $c$ -stars near the nodes of the galactic rotation term, we find  $|\overline{\delta\rho}| = \pm 12$  km/sec for  $cB$ - $cA5$ ,  $|\overline{\delta\rho}| = \pm 8$  km/sec for  $cA8$ - $cG4$  and  $|\overline{\delta\rho}| = \pm 11$  km/sec for  $cG5$ - $cM5$ . We can expect high accuracy of a group mean

absolute magnitude only for stars with a mean rotational term,  $\bar{rA}$ , greater than 20 km/sec.

The mean absolute magnitude for stars selected by apparent magnitude will be affected by various statistical errors of the form  $(\log \bar{x} - \log x)$ . The distribution of the velocities is skew, as is the distribution of the distances of stars selected by apparent magnitude. Interstellar absorption and increasing  $\bar{rA}$  both decrease these statistical corrections for the distant stars. The corrections have been estimated as of the order of +0.25 mag for a modulus of 6 mag, and +0.10 mag for a modulus of 15 mag. The mean absolute magnitude is obtained from

$$\bar{M} = \bar{m} - \overline{\text{Absorption}} - 5 \log \left( \frac{\rho}{\sin 2(l - l_0) \cos^2 b} \right) + 5(\log A + 1 + [\log \bar{r} - \overline{\log r}]). \quad (6)$$

The major uncertainty arises from the mean interstellar absorption term in (6). For the *B*-stars the mean absorption can be evaluated from the photoelectric color excesses, which were very kindly supplied by Professor Stebbins before publication.<sup>1</sup> For visual light the ratio<sup>2, 3</sup> of absorption to color excess,  $E$ , has been taken as seven. No suitable measures of color excess of the later spectral types exist, and a mean coefficient of absorption derived from the *B*-stars has been used.

A value of the constant,  $A$ , based on secular parallaxes and radial velocities has been given by Plaskett and Pearce.<sup>4</sup> Because of the large range in the luminosities of the *B*-stars it was necessary to introduce small changes in certain of the statistical corrections used by Plaskett and Pearce, especially in  $\bar{\pi} \cdot \bar{r}$ . The derived value of 17 km/sec/kpc differs very little from their value of 15.5 km/sec/kpc.

The absolute magnitudes of the *B*-stars have recently been determined by Whitford<sup>5</sup> from radial velocities, and I am indebted to him for discussions of these results. A different statistical treatment may lend some interest to my new determination. Only stars for which  $\sin 2(l - l_0) \cos^2 b \geq 0.5$  were used. The solar motion was taken constant for all stars, and equal to 20 km/sec with the apex at  $18^\circ$ ,  $+28^\circ$ . The  $K$ -term may be neglected if the stars are uniformly distributed over both positive and negative maxima of galactic rotation. The velocities of the interstellar lines were used, when available,<sup>6</sup> as well as the stellar velocities. The spectral classifications are of mixed origin, coming from Mt. Wilson, Victoria and the *Henry Draper Catalogue*.

Proper weighting criteria are difficult to establish. If the dispersion in absolute magnitude were small, a grouping by  $m' = m - 7E$  would be a grouping by distance, and the weights would be measured by the mean rotational term,  $\bar{rA}$ . In view of the large dispersion, and the effects of



absorption, a grouping by  $m'$  favors intrinsically faint stars. A grouping by the values of the rotational term unduly favors stars of large peculiar motion. Both methods have been used, and the results are given, with the mean values, in table 1. The column (m. e.) gives the estimated mean error of the mean absolute magnitude arising from the peculiar velocities of the stars. Included in table 1 are the mean absolute magnitudes  $M_*$  obtained from the Victoria secular parallaxes<sup>4</sup> by Stebbins, Huffer and Whitford,<sup>5</sup> who corrected for the mean interstellar absorption of stars of a given magnitude and spectral type. Since the latter is based on Victoria classifications, no distinction can be made for the  $c$ -stars, and  $M_*$  may be systematically too bright.

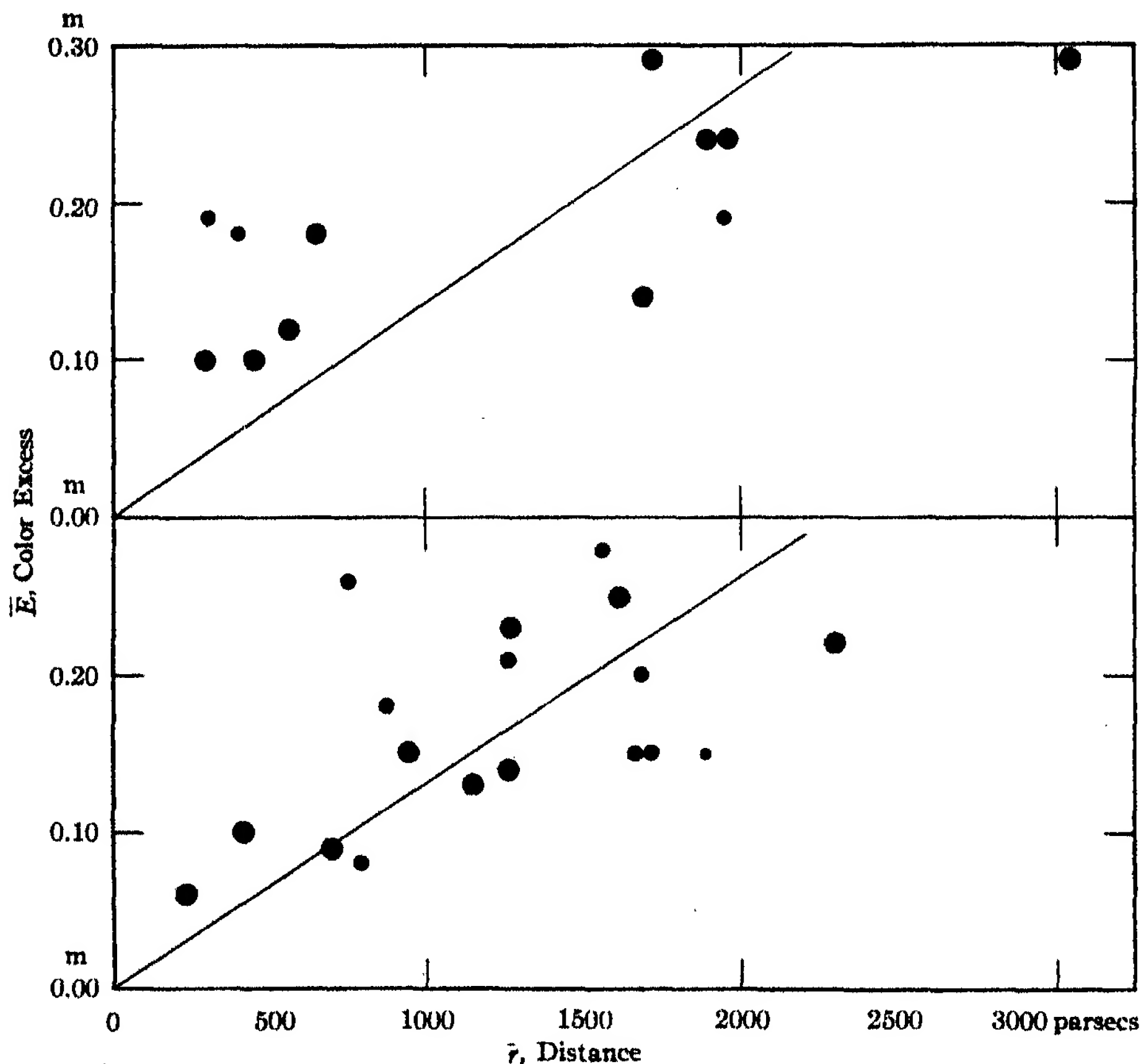
TABLE 1  
EARLY-TYPE STARS OF KNOWN COLOR EXCESS

TYPE	$\overline{rA}$ KM/SEC	$n$	$(m')$	$\overline{M}$ ( $rA$ )	MEAN	M. E.	$M_*$
$cB, cA$	29	48	$-6^M1$	$-6^M4$	$-6^M3$	$\pm 0^M2$	..
05-09	20	35	-4.2	-4.5	-4.4	$\pm 0.3$	-4.6
B0	20	34	-4.4	-4.7	-4.6	$\pm 0.3$	-4.0
B1	15	23	-3.6	-4.1	-3.9	$\pm 0.4$	-3.7
B2	12	63	-2.8	-3.8	-3.3	$\pm 0.3$	-3.1
B3	6	150	-0.7	-1.3	-1.0	$\pm 0.5$	-1.7

The differences between the absolute magnitudes in table 1 and those found by Whitford<sup>5</sup> are small, and arise mainly from the smaller value of the rotational constant adopted. The value for B3 is of low weight, since the systematic errors of the method are most serious for these near-by stars. The high mean absolute magnitude found for the early supergiants is of significance in problems of interstellar reddening. The great number of highly reddened stars among the apparently bright  $B$ -stars indicates the large number of supergiants. In an earlier spectrophotometric survey I have measured<sup>2</sup> gradients of 38 reddened  $B$ -stars. Of these 38 stars it is now known that at least 16 are supergiants; they have been so identified from the Mt. Wilson classifications, from high observed rotational terms and from unpublished data by O'Keefe, at the Yerkes Observatory. The systematically smaller integrated hydrogen-line absorption in the violet for the supergiants would at least partly explain a small deviation from linearity of the relative gradients found in that investigation.

The general widening of the scale of luminosities is also of importance in the derivation of mean values of the interstellar absorption coefficients. For example, the use of the conventional scale of luminosities (B0 near  $-3^M5$ ) in the earlier work of Stebbins and Huffer<sup>6</sup> resulted in a mean photoelectric reddening of 0.28 mag/kpc. If distances based on galactic rotation alone are used the resultant coefficient is much lower. Figures 1 and 2 show the photoelectric color excess of 177 of the stars of table 1 as a function

of distance determined from the radial velocities. The stars have been grouped by spectral type, and arranged in order of distance by ( $rA$ ) in figure 1, and by apparent magnitude corrected for reddening ( $m'$ ) in figure 2. A least-squares solution, forced through the origin, gives a mean reddening of 0.13 mag/kpc for both groupings. This coefficient refers to a range of



FIGURES 1 AND 2

The mean color excess,  $\bar{E}$ , is plotted against distance determined from galactic rotation. In figure 1 (*top*) the stars are grouped by  $rA$ ; in figure 2 (*bottom*) by  $m'$ . The lines represent least-squares solutions, with a reddening coefficient of 0.13 mag/kpc.

longitude,  $340^{\circ}$ – $40^{\circ}$ ,  $70^{\circ}$ – $130^{\circ}$  and  $160^{\circ}$ – $220^{\circ}$ , and to galactic latitudes less than  $\pm 5^{\circ}$ . The use of distance moduli based on the present scale of absolute magnitudes should result in a similar coefficient, since the moduli are compatible with distances derived from galactic rotation. The reddening of 0.13 mag/kpc can be combined with the  $\lambda^{-1}$  law for interstellar red-

dening to yield a mean photographic absorption coefficient of 1.2 mag/kpc, in agreement with Joy's value based on the galactic rotation of the Cepheids.<sup>7,8</sup> Selection effects in the data must be seriously considered, and the dependence of  $\bar{E}$  on  $\bar{r}$  in figures 1 and 2 is not such as to establish a constant value of the absorption coefficient.

Lists suitable for a calibration of the later-type stars designated as spectroscopically highly luminous can be obtained from the *c*-stars in the catalogs of Mt. Wilson spectroscopic absolute magnitudes<sup>9, 10</sup> and from a list of *c*-stars by Miss Payne.<sup>11</sup> From the Mt. Wilson list I have also selected those *M*-stars for which the spectroscopic absolute magnitude,  $M_{sp}$ , was brighter than  $-1^m.0$ . The material available is too small for a satisfactory statistical analysis, but the results collected in table 2 may serve as a preliminary calibration. The derived mean absolute magnitudes are given both before and after correction for interstellar absorption. The correction

TABLE 2  
LATER-TYPE *c*-STARS

SOURCE	TYPE	$\bar{rA}$ KM/SEC	$n$	$\overline{[b]}$	$\bar{m}$	$\bar{M}$ NO ABS.	ABS.	M. D.
Mt. Wilson	A8-G4	15	24	9°	4.89	$-4^m.7$	$-5^m.1$	$\pm 0^m$
	G5-K8	5	16	8	5.20	-2.0	-2.2	$\pm 2.4$
	M0-M5	4	9	7	4.70	-2.3	-2.4	$\pm 4.0$
$M_{sp} \leq -2^m.5$	A8-M0	13	12	9	4.78	-4.4	-4.8	$\pm 0.9$
All	A8-M5	10	49	8	4.96	-3.9	-4.2	$\pm 0.6$
Mt. Wilson; Payne	B8-A5	21	22	9	5.43	-4.8	-5.4	$\pm 0.5$
Payne	F0-G5	12	18	12	4.57	-4.5	-4.9	$\pm 0.7$
Morgan Lum. Class Ia.	F4-F8	26	4	4	3.90	-6.9	-8.2	$\pm 0.8$

for absorption has been taken as that derived from the *B*-stars, 0.93 mag/kpc, in low galactic latitudes. I have arbitrarily adopted the value of 0.50 mag/kpc for the stars in higher mean galactic latitudes, with suitable interpolated values. The stars of the Mt. Wilson lists have been analyzed in groups according to spectral type, and a group of the most luminous stars has also been treated, with  $M_{sp} \leq -2^m.5$ . It is apparent that the earlier stars, from *cB8* to *cG5* possess high mean luminosity, near  $-5^m$ . Results for the later stars are very uncertain, and suggest that they do not form a homogeneous group. The mean absolute magnitude for this selection of *cB8-cA5* stars,  $-5^m.4$ , is not far from that of the *cB* and *cA* stars of table 1,  $-6^m.3$ . An interesting result is obtained for a group of four stars which Dr. Morgan has kindly selected for me as spectroscopically the most luminous of the brighter stars. They are  $\rho$  Cas,  $\varphi$  Cas,  $\epsilon$  Aur and  $\delta$  CMa; they have the amazing rotational term of  $\bar{rA} = 26$  km/sec, and yield the mean absolute magnitude of  $-6^m.9$  without correction for interstellar ab-

sorption. I am indebted to Dr. Morgan and to Dr. Keenan for discussions of the problems of the calibration of spectroscopic luminosities.

While these results are of a preliminary nature, it is apparent that spectroscopic absolute magnitudes of the supergiants can be accurately calibrated, even if few stars are available. For a calibration of a group of ten stars within  $\pm 0^m.5$  a mean distance greater than one kiloparsec is necessary; for supergiants this requirement would be fulfilled for stars fainter than the sixth magnitude. The errors of the measured radial velocities need only be less than the velocity dispersion, that is, less than  $\pm 8$  km/sec.

<sup>1</sup> Stebbins, Huffer and Whitford, *Ap. J.*, 91, 20 (1940).

<sup>2</sup> Greenstein, *Ibid.*, 87, 151 (1938).

<sup>3</sup> Stebbins, Huffer and Whitford, *Ibid.*, 90, 459 (1939).

<sup>4</sup> *Pub. Dom., Ap. Obs. Victoria*, 5, 289 (1936).

<sup>5</sup> Merrill, Sanford and Burwell, *Ap. J.*, 86, 205 (1937).

<sup>6</sup> *Pub. Washburn Obs.*, 15, Part 5 (1934).

<sup>7</sup> *Ap. J.*, 89, 271 (1939).

<sup>8</sup> Van Rhijn, *Gron. Pub.*, 47 (1936).

<sup>9</sup> Adams, Joy, Humason and Brayton, *Ap. J.*, 81, 187 (1935).

<sup>10</sup> Merrill, *Ibid.*, 81, 351 (1935).

<sup>11</sup> *Stars of High Luminosity*, Appendix A (1930).

## SYNTHETIC SPECTRA FOR SUPERNOVAE

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*Introduction.*—The excellent series of spectra of the supernovae in I. C. 4182 and N. G. C. 1003, published by Minkowski,<sup>1</sup> present for the first time a basis for a quantitative interpretation. Eight typical spectra, showing the major stages of the development during the first two hundred days, are shown in figure 1; they are directly reproduced from Minkowski's microphotometer tracings, with some smoothing for plate grain. The pre-maximum spectra (which show few features in addition to an apparent continuum) and the very late spectra (more than two hundred days after maximum) are not represented. Since all supernovae apparently show nearly identical spectral changes,<sup>2</sup> this series can be considered as representative of all, both with regard to the essential spectral features and to changes with time. The broad features in the blue-violet region appear to shift to the red with time, while the features in the red region show variations but no systematic shift in wave-length.

A comparison of Minkowski's near-maximum spectra (not shown here) with his spectra of  $\zeta$  Aquilae (B9n) shows relatively more energy in the red and yellow regions of the supernova spectrum. Since the latter spectrum is apparently nearly continuous at this stage, an obvious conclusion is that it arises from a relatively low-temperature source (probably less than  $12,000^\circ\text{K}$ ). A possible alternative is that the spectrum consists of exceedingly broadened bright lines of high excitation, which happen to coalesce and simulate black-body radiation at a low temperature. Other alternatives are even less plausible. The working hypothesis of a low temperature continuum is suggested also by analogy with the spectra of ordinary novae, where temperatures of  $8000^\circ$  to  $10,000^\circ\text{K}$  are observed<sup>3</sup> in the early stages, at which time no strong absorption or emission spectra are present. It is supported strongly by the only lines that have been positively identified in supernova spectra: the narrow emission lines of [O I] at  $\lambda\lambda$  6300 and 6363, which appeared in the spectrum of the supernova in I. C. 4182 between 158 and 184 days after maximum. We regard these two lines as interstellar lines produced in the neighborhood of the supernova by the pulse of high-intensity energy emitted near maximum light. A similar suggestion has been made by Zwicky.<sup>4</sup> These nebular lines of neutral oxygen originate in a transition that goes to the ground state of the neutral atom, and are thus of the lowest excitation possible for atomic emission.

A few days after maximum light, the spectrum of a supernova begins to show more detail, but no sharp lines ever appear, with the exception of the oxygen lines just mentioned. If an emission or absorption spectrum exists, the lines must be greatly broadened, with a width (if interpreted as a Doppler effect) corresponding to a velocity probably of the order of 6000 km./sec. in both approach and recession.<sup>5</sup> The steepness of the violet edge of the feature near  $\lambda$  5890 suggests that the velocity does not greatly exceed this value. Emission lines broadened by  $1/25$  of their wave-length would not be separately identifiable unless their centers were separated by at least 150 Å., the separation necessary depending completely on the nature of the profiles; the case for square profiles has been discussed elsewhere.<sup>6</sup> The assumption of broadened lines has a precedent in the interpretation of the spectra of ordinary novae, where the broadening is universally attributed to Doppler effect in an expanding stellar atmosphere. The analogies between supernovae and novae have been discussed by one of the writers<sup>7</sup> and by Zwicky;<sup>8</sup> in the present paper we shall assume that a rough parallel is, in principle, a legitimate one.

The most common emission spectrum observed astronomically is the Balmer series of hydrogen; if these lines were present in the spectra of supernovae with the relative strength that they usually show in the spectra of novae, we should expect to find broad maxima near their positions.



As such maxima are not conspicuous (if indeed they are present at all) in the earlier stages of supernovae, it must be concluded that the hydrogen spectrum is not strong at this phase. Such a conclusion may seem incompatible with the assumption of a relatively low temperature; the element hydrogen, however, while it is known to be the main constituent of stellar atmospheres,<sup>9</sup> is only fairly abundant in stellar interiors.<sup>10</sup> The amount of energy and matter involved in the supernova outburst must necessarily be enormous in comparison even with those of the ordinary nova outburst. We are here dealing chiefly with matter from the interior of the star and not from its exterior. Consequently the relative weakness of the hydrogen lines is not very surprising.<sup>11</sup>

Two general features of the supernova spectra suggest a great abundance of helium: the only apparently permanent feature of the spectrum, near  $\lambda$  5875 (the  $D_3$  line of He I); and the later maximum in the blue, which probably contains  $\lambda$  4686 (the first line of the Fowler series of He II), as well as the group of N III lines near 4640. These lines are important contributors to the spectra of ordinary novae.

1. *Procedure.*—In view of all the above considerations, it has seemed worth while to attempt to reconstruct the supernova spectra by summation of the bright-line spectra of the astrophysically commoner elements, in the stages of ionization for which the data are reasonably complete. The adopted procedure, which is to be described in detail in a forthcoming paper,<sup>12</sup> is summarized in the following paragraphs.

(a) *The Line Intensities.*—Determinations were made of the relative intensities of the permitted lines within each of the following nineteen spectra, over the wave-length range  $\lambda$  3000 –  $\lambda$  6700: H I, He I, He II, C II, C III, C IV, N II, N III, N IV, N V, O II, O III, O IV, O V, O VI, Na I, Ca II, Fe II and Fe III. Within series spectra, well-separated multiplets and supermultiplets, in cases where present theory is readily applicable, the relative intensities of lines (or multiplets) were calculated by application of the sum rules and by wave-mechanical theory. In all cases  $LS$  coupling was assumed. For the evaluation of the Boltzmann factors, temperatures roughly appropriate to the degree of ionization (15,000°K to 100,000°K) were assumed. In several cases, laboratory intensities (supplemented for Fe II by astrophysical intensities) were used to adjust the relative values between multiplets or supermultiplets, because the theory was uncertain or inapplicable. In all cases where they were available, the laboratory values furnished a satisfactory check on the theoretical intensities.

(b) *The Line Profiles.*—The line profiles were assumed to be parabolas with their major axes vertical and their vertices upwards; the intercepts on the wave-length axis were symmetrically placed about the line center, and separated by a distance equivalent to a velocity of 12,000 km./sec. The areas were made proportional to the calculated intensities. A profile



with steep sides and a rounded top was chosen primarily from an inspection of the supernova spectra, particularly in the visual regions; this general shape is often shown by the bright lines of the Wolf-Rayet stars and novae. The parabolic form was adopted for convenience in drawing and calculation. It is clear that truly symmetrical lines are not to be expected from an expanding atmosphere; but, for example, in some of the cases discussed by Chandrasekhar for outwardly accelerated motion,<sup>13</sup> the deviations from symmetry are not great.

(c) *Integration of Individual Spectra.*—The parabolic profiles of the lines (or groups of lines) of each individual atom were drawn on a prismatic dispersion scale proportional to that used by Minkowski<sup>1</sup> (spectrographs *e* and *f*). Where the lines overlapped, the intensities were summed at small intervals in wave-length. The effects of overlapping on the final summed spectrum are difficult to foresee, and are sometimes surprising.

TABLE 1  
COMPOSITION OF SYNTHETIC SPECTRA

ATOM	A ≡ I %	B %	C %	II %	III %
H I	5.2	6.9	..	4.8	4.6
He I	7.8	15.6	..	8.7	9.4
He II	..	9.6	16.9	5.2	9.2
C II	..	18.2	..	4.9	8.7
C III	..	..	23.2	3.6	6.3
N II	..	24.3	..	6.6	11.6
N III	..	..	57.2	8.7	15.4
O II	..	8.6	..	2.3	4.1
O III	..	..	2.7	0.4	0.7
Na I	2.3	..	..	1.4	0.6
Ca II	5.9	..	..	3.4	1.5
Fe II	78.8	..	..	45.4	20.0
Fe III	..	16.8	..	4.5	8.0

(d) *The Final Integrations.*—In order to produce combined spectra of successively increasing excitation, the individual spectra were summed in groups designated *A*, *B* and *C* in order of excitation. The percentage of the energy with wave-length greater than  $\lambda$  3800 contributed by each atom to the combined spectrum is given in table 1. The percentages were, of course, chosen arbitrarily, on the basis of the observed spectra of novae and supernovae. The contribution from hydrogen was made very small, and might even have been omitted altogether without affecting the results appreciably.

Spectrum *A* was combined with a strong continuum, corresponding to a temperature of 10,000°K.; spectrum *B*, with a moderately strong continuum corresponding to a temperature of 16,000°K.; and spectrum *C*, with a weak continuum corresponding to a temperature of 28,000°K.

Because our synthetic spectra were to be compared with Minkowski's published tracings, corrections for plate sensitivity, absorptions and prismatic dispersion were applied to the calculated spectra. These corrections were deduced from Minkowski's published tracings of a series of spectra of  $\zeta$  Aquilae (made with spectrograph *f* on "Agfa Supersensitive Panchromatic" film), on the assumption that the energy distribution in the star's spectrum corresponds to black-body radiation at  $12,000^{\circ}\text{K}$ .

Spectrum *A*, corrected by the procedure described in the last paragraph, is shown, as predicted spectrum I, in figure 1, for comparison with the 9- and 10-day spectra of the supernova in I. C. 4182, drawn below it.

Spectrum II, compared in figure 1 with the 20- and 24-day spectra of the supernova in I. C. 4182, is of a somewhat higher mean state of excitation than Spectrum I, being compounded from spectra *A*, *B* and *C* and their respective continua in the proportions (bright line energy): *A*, 58%; *B*, 27%; *C*, 15%. The fifth column of table 1 shows the resultant percentages of energy contributed by the individual atomic spectra.

Spectrum III, compared in figure 1 with the 44- and 51-day spectra of the supernova in N. G. C. 1003, and with the 67- and 136-day spectra of the supernova in I. C. 4182, is similarly compounded in the proportions: *A*, 25%; *B*, 48%; *C*, 27%; the atomic contributions are shown in the sixth column of table 1.

2. *Discussion.*—In comparing Minkowski's microphotometer tracings and our spectra, it is important to notice several things. First, his tracings are made from unwidened spectra, analyzed with a relatively long microphotometer slit, and they have not been reduced to relative intensities. With such an arrangement the effects of "burning out" (noticeable especially in the red regions) are particularly difficult to predict and allow for, because the characteristic curve of the emulsion will be different from those ordinarily encountered in spectrophotometry. Accordingly, no attempt has been made to correct our predicted spectra for the characteristic curve. Secondly, the material permits only an approximate determination of the sensitivity of the emulsion, which may vary appreciably over a period of several months. For both these reasons the conspicuous peak at the red end of the predicted spectra should not be considered as more than an indication of relatively high red energy. In most of Minkowski's spectra of the early stages, the red end is certainly "burned out;" in the cases where it is not (supernova in I. C. 4182 at 41 days; supernova in N. G. C. 1003 at 29 and 44 days, the latter spectrum being shown in figure 1), the excessive strength at the red end is quite obvious, and similar to that shown in the predicted spectra.

The extent to which the predicted spectra resemble the observed ones may be seen from inspection of figure 1. The strange phenomenon, noted by Minkowski, that the blue regions of the spectrum show a progressive

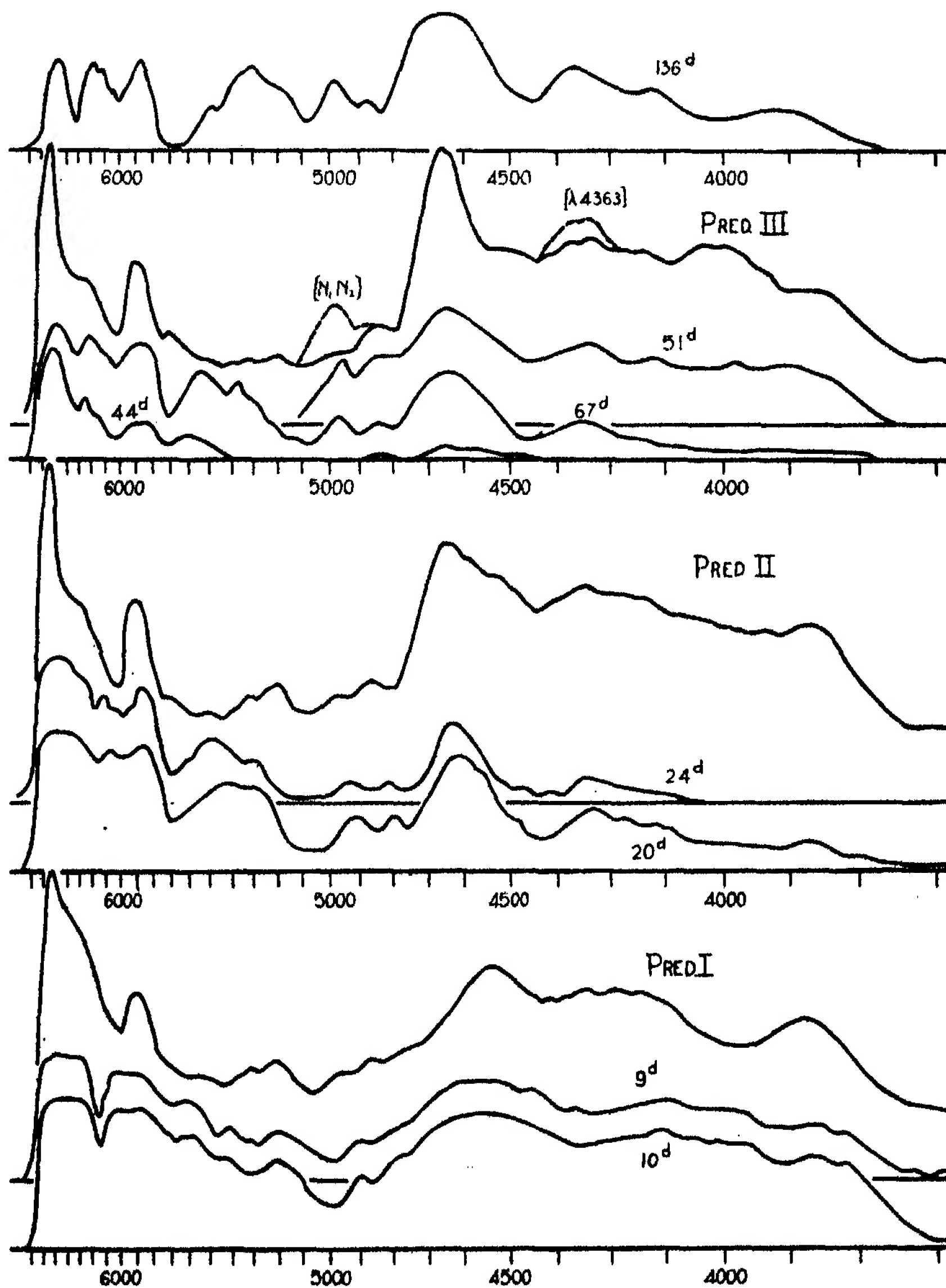


FIGURE 1

Observed and predicted spectra of supernovae. The observed spectra are of SN IC 4182 with the exception of those at 44<sup>d</sup> and 51<sup>d</sup> after maximum light, which are of SN NGC 1003.

red shift with time, which the yellow and red regions do not share, is also displayed by the predicted spectra. Apparently this phenomenon can be explained merely as the result of changes in the atomic makeup of the spectrum, resulting from increase of excitation with time. It is also possible for a small red shift to occur as a result of a broad line or group of lines falling in a region where emulsion sensitivity varies rapidly with wavelength; a change with time of the intensity of the underlying continuous background produces a shift, which may be of the order of fifty Å. U.

It will be seen from table I that comparatively few atoms have been used in forming the synthetic spectra. Relatively small changes in the relative intensities of spectral lines or in the assumed line breadths or profiles may produce marked changes in the integrated spectra, particularly with regard to detailed features. It is, therefore, not surprising that there are discrepancies between the predicted and observed spectra. It can be seen that the addition of the more likely "forbidden" lines which might well be present (the auroral and nebular lines of O III,  $\lambda\lambda$  4363, 4959, 5007) will improve the agreement between observation and prediction. These lines are inserted with broken curves in Spectrum III of figure 1. The commonly occurring "forbidden" lines that were omitted were the auroral lines ( $\lambda$  5755) of N II and ( $\lambda$  5577) of O I which seem not to be present, the corresponding nebular pair ( $\lambda\lambda$  6548, 6584) of N II, and ( $\lambda\lambda$  6300, 6363) of O I, the two latter pairs occurring in a part of the spectrum that is already very intense. The "forbidden" lines of Fe II were omitted because of difficulty in predicting the intensities; their positions are such as not greatly to affect the agreement. The same is true for the "forbidden" lines of Fe III.

The early minimum at  $\lambda$  6140 appears to be an absorption feature rather than a gap between emission lines; it disappears relatively soon (between 25 and 40 days after maximum). In view of the strong evidence for relatively low temperatures and the fact that there may be a temperature gradient in the atmospheres of supernovae, it seems quite possible that this feature, and perhaps some other features of the early spectra, may arise from molecular absorption in the outer levels of the atmospheres. The strong absorption band of TiO, with a head at  $\lambda$  6159, would produce an absorption feature at almost precisely the observed position after allowance is made for a velocity of approach. A study of Öhman's paper on the red spectra of the cool stars<sup>14</sup> suggests, however, that other TiO bands, incompatible with the observed features of supernova spectra, might then be expected in the blue-green regions.

Another feature that may possibly be of molecular origin is the wide minimum at  $\lambda$  3800, which is best shown in Popper's spectra of the supernova in I. C. 4182, taken in the early stages.<sup>15</sup> This minimum is strongly reminiscent of the CN absorption shown so conspicuously by Nova Herculis a few days after maximum.

There is at the present time no consistent interpretation for the powerful emissions around  $\lambda$  5300 in the later stages, or for the minimum near  $\lambda$  6300 that occurs about a hundred days after maximum; however, at these wave-lengths the atomic spectra are relatively less well known, and it is also possible that higher excitation of well-known elements may be complicating the problem.

The spectra of C IV, N IV, N V, O IV, O V and O VI studied by the writers provide a conspicuous maximum in the blue, similar to that observed in the later stages of the supernovae. They are not included in the present discussion, however, because the agreement with observations in the other spectral regions is poor.

Although not shown in figure 1, the integrations have also covered the ultra-violet regions down to  $\lambda$  3000, and indicate that under the observational conditions the almost complete absence of visible features of wave-lengths shorter than  $\lambda$  3600 is exactly what would be predicted.

The tentative physical picture of a supernova outburst, consistent with the present discussion, bears a general resemblance to the accepted picture of a nova outburst. An enormous expulsion of matter from a star's exterior occurs within a relatively short time, accompanied, of course, by extremely high temperatures at low levels of the effective atmosphere. As seen externally, however, the various processes of absorption and emission serve to transform the high-temperature radiation to a continuum of low effective temperature. As the total rate of radiation decreases with time, the level of the effective photosphere in the semi-transparent atmosphere drops rapidly, and an increase in the observed effective temperature occurs. A large temperature gradient exists in the atmosphere, so that the distribution of radiation deviates more and more from any semblance of black-body radiation,<sup>16</sup> the strength of the continuum decreases and emission lines of widely different states of excitation are simultaneously observed. Because of the rapid drop in total radiation, the large physical dimensions (many astronomical units) of the atmosphere, and possibly also because of an only moderate rise in effective temperature, a condition develops that more nearly resembles Wolf-Rayet emission than a late-stage nova emission. Forbidden lines possibly may not predominate at any stage of a supernova outburst.

The remarkable similarity of the spectra of various supernovae (as compared with the dissimilarity among various novae), the probable low abundance of hydrogen (and possibly of oxygen) and the high abundance of helium and iron suggest a uniformity among the stars that become supernovae. An interpretation is difficult at the present time. The relative importance of helium, carbon and nitrogen in the spectra of supernovae is of especial interest because of Bethe's recent theory<sup>17</sup> of stellar energy generation based on a carbon-nitrogen (hydrogen) chain of atomic transformations.



The low packing fraction of iron and the apparent abundance of the element in the supernovae seem possibly to be related.<sup>18</sup>

The authors are especially indebted to Dr. L. Goldberg for advice with regard to the theory of line intensities, and to Drs. B. Edlén and P. Swings for use of their unpublished data on the spectrum of Fe III.

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## MICELLE FORMATION IN AQUEOUS SOLUTIONS OF DIGITONIN

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(1) The well-known experiments of McBain and his collaborators<sup>1</sup> established that micelles are formed in aqueous solutions of electrolytes such as soaps and other paraffin-chain salts, particularly those of the sulphonic acids.<sup>2</sup> The methods of detecting micelle formation have usually consisted in showing that measurements of conductivity, freezing point, dew point, etc., deviate from those predicted for the individual ions. Since such methods portray only the average behavior of many particles, they have yielded no information regarding the size or quantity of the micelles in solution.

Using the ultracentrifuge to investigate particle size, we have found that a nonelectrolyte, the glucoside digitonin, forms large micelles in aqueous solution. It is known that the digitonin molecule possesses a



hydrophobic nucleus similar to that of the sterols, and several carbohydrate side-chains of hydrophylic nature. It is probable that micelle formation may occur in aqueous solutions of many other substances which are partly hydrophobic and partly hydrophylic.

(2) The digitonin was obtained from Eimer and Amend, New York, as "crystalline digitalin" and should not be confused with the true digitalin,

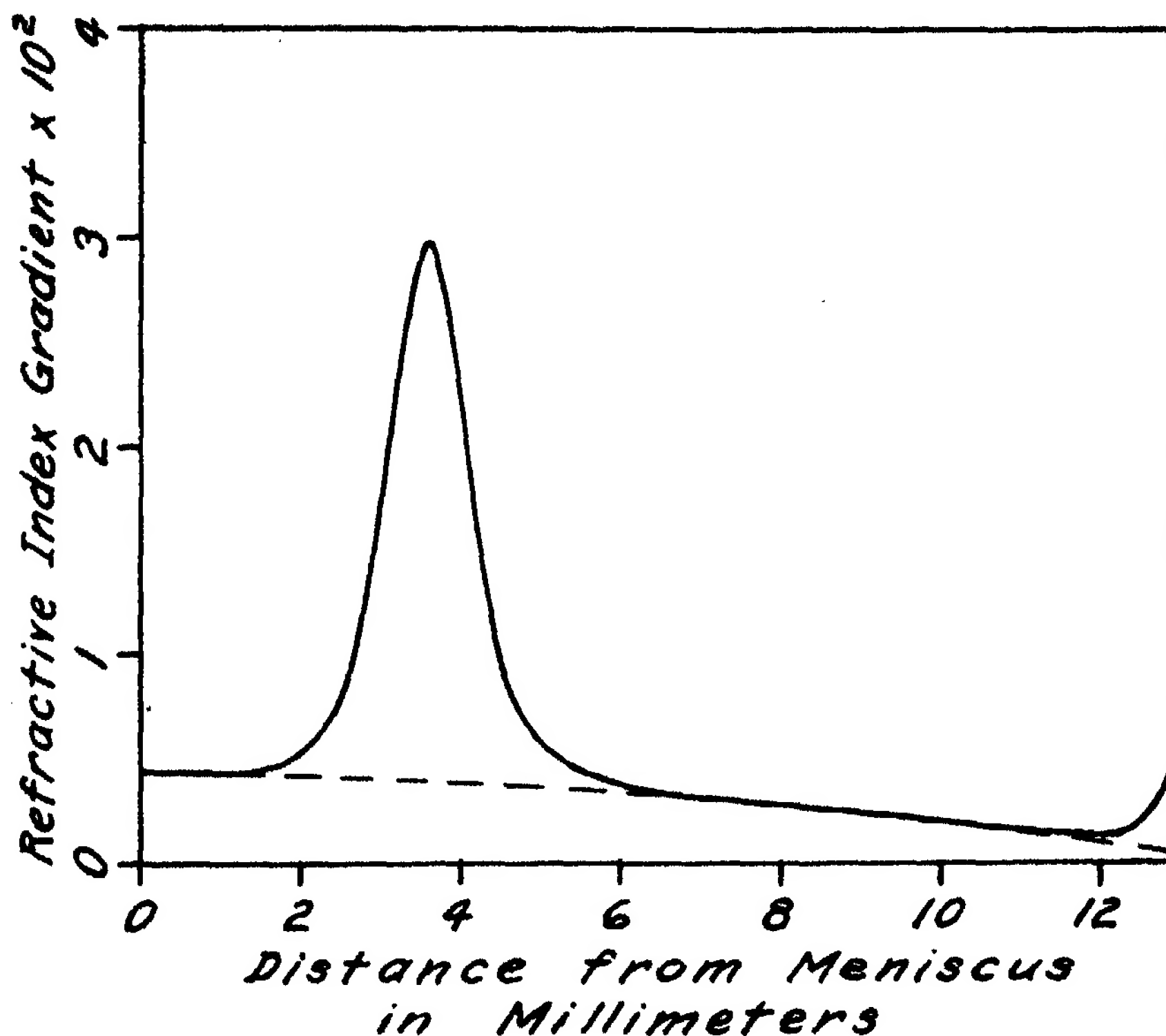


FIGURE 1

Sedimenting boundary of 2.5 per cent digitonin after 1 hour of centrifugation at 780 revolutions per second. The dotted line indicates the base line for water at the same speed.

a cardiac glycoside. The preparation used in this study gave the familiar digitonide precipitation reaction when it was added in alcoholic solution to cholesterol. For centrifugation the digitonin was dissolved in distilled water by slowly heating to boiling, and then cooling to room temperature. A 5 per cent solution showed only a faint opalescence.

All of the measurements were made using the air-driven ultracentrifuge

of Bauer and Pickels.<sup>3</sup> The sedimentation velocity of the digitonin was determined by centrifuging at 780 revolutions per second, which was equivalent to an average force of 160,000 gravity. Observations of the sedimentating material were made by a direct-reading refractive index method designed especially for the ultracentrifuge and utilizing a scanning system similar to that described by Longsworth<sup>4</sup> for electrophoresis measurements. The distribution of the micelles was recorded at 20-minute intervals by photographing the refractive index diagram. The runs were carried out at temperatures in the neighborhood of 25°C.

Figure 1 shows a tracing of a magnified refractive index photograph made with a 2.5 per cent digitonin solution 60 minutes after the centrifuge was brought to full speed. All of the photographs have shown only one discrete sedimenting boundary which was characteristic of an approximately homogeneous group of particles.

The sedimentation velocity of the micelles was estimated from curves such as the one illustrated by measuring as a function of time the successive displacements from the meniscus of the peak, which corresponds to the mean position of the diffuse boundary. Using the values for the viscosity and density of water and correcting the data to 20°C., the sedimentation constants were computed from the sedimentation velocities. Six independent determinations gave the following values: 5.35, 6.15, 5.33, 5.67, 6.36 and  $6.41 \times 10^{-13}$  cm. sec.<sup>-1</sup> dyne<sup>-1</sup>. These yield an average value for  $S_{20}$  of  $5.88 \times 10^{-13}$  cm. sec.<sup>-1</sup> dyne<sup>-1</sup> with an average deviation of less than 7 per cent.

One of the runs was made with 0.63 per cent digitonin; its sedimentation constant lies within the range of the other determinations which were made with 2.5 per cent solutions. Freshly prepared solutions or those several weeks old gave similar values. Some of the observations were made while studying the effect of digitonin on the chloroplast protein of spinach; no correlation was found between the sedimentation velocity of the digitonin micelle and the protein concentration, which varied up to about 1 per cent, even though the chlorophyll migrated together with the digitonin micelles.

(3) The concentration of material sedimenting at a measured rate can be estimated by measuring the area under the respective refractive index curve, if the refractive indices of solution and solvent are known. The differential refractive index, and hence the concentration, is directly proportional to the area. The solutions used were all originally made up to a concentration of 5 per cent. With one of these solutions, a refractive index determination was made which in itself had no significance as an absolute value since the material was of unknown purity and contained some water of crystallization. However, this determination could be used for estimating the relative concentration of the sedimenting material since the same solution was used for both measurements.

The refractive index of the 5 per cent solution at 20°C. was 1.3402; that of water measured with the same refractometer was 1.3328. Therefore, the differential refractive index of 2.5 per cent digitonin was 0.0037. Measurements on the photographs taken during centrifugation indicated a differential refractive index of 0.00368 for the sedimenting boundary. This shows that in a solution of this concentration practically all of the

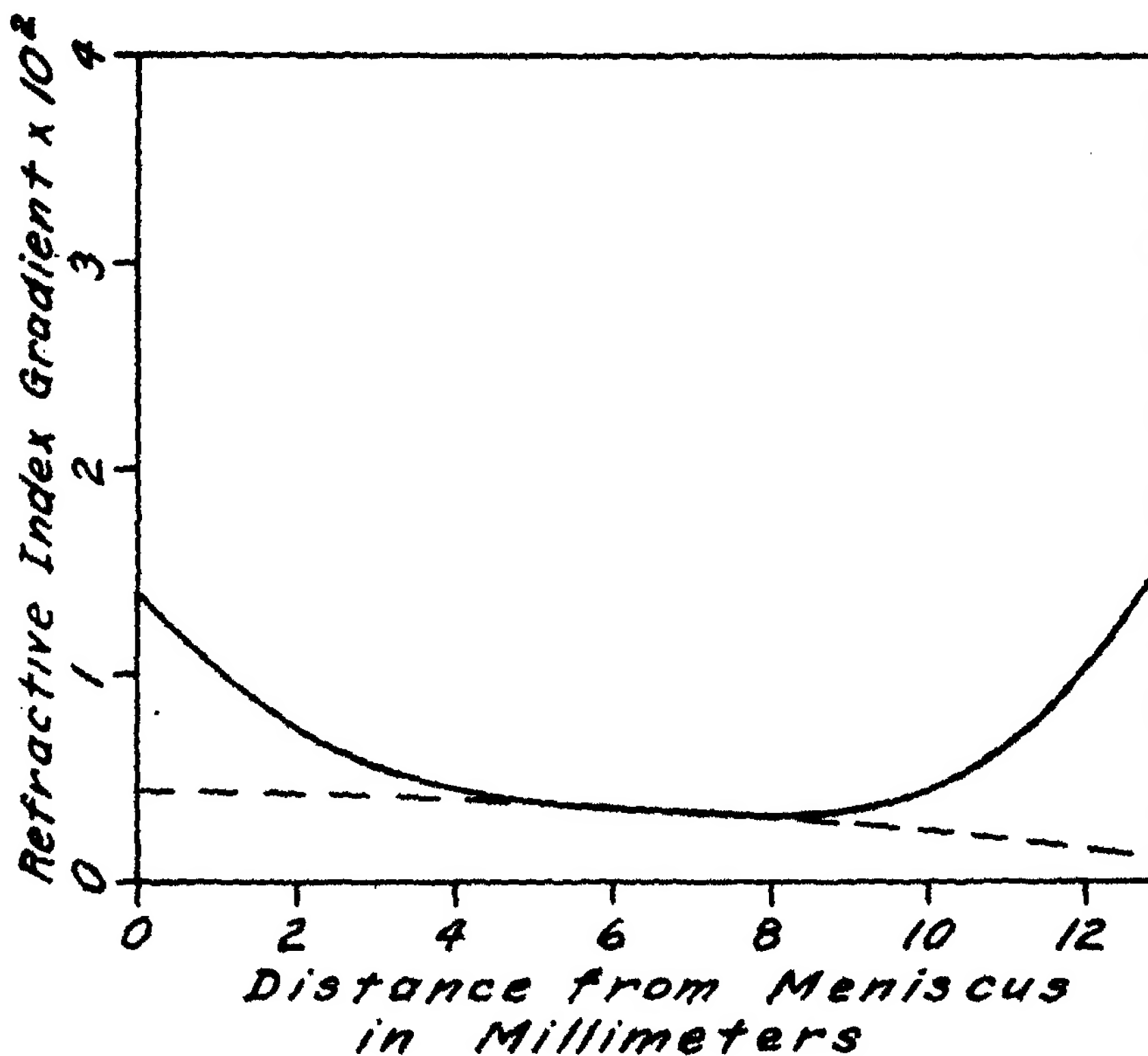


FIGURE 2

Refractive index curve of 5 per cent sodium desoxycholate after centrifuging for 90 minutes at 780 revolutions per second.

digitonin molecules are in the form of micelles of only one well-defined size. Nevertheless, there must be an equilibrium between these large micelles and a few smaller particles, probably the individual molecules, since the digitonin can be completely dialyzed through a cellophane membrane which will not permit the passage of particles even a tenth the probable size of these micelles.

(4) The exact micellar size cannot be computed because the shape and

density of the particles are unknown. A minimum value of the micellar weight can be obtained by assigning a maximum possible density value and applying Stokes' law. Since approximately half of the digitonin molecule is lipoidal in character, the average density is quite unlikely to be higher than that of most proteins, i.e., 1.33. With this value as an upper limit, the micellar weight is computed to be approximately 75,000. Since the molecular weight of digitonin is 1228, the micelles are extremely large, involving at least 60 of the primary molecules. The true micellar weight is undoubtedly larger than 75,000 since hydration, a lower density or any deviation from spherical shape would yield a larger size as computed from the observed sedimentation constant.

From the shapes of the refractive index curves of the sedimenting micelles, it is possible to determine the homogeneity of the particles and, if homogeneous, to obtain an approximate diffusion constant. The curves obtained were nearly symmetrical about the peak positions throughout the centrifugation, and showed a smaller spread than that expected for a micellar weight of 75,000. This shows not only that the particles are homogeneous, but that the true micellar weight must be higher than 75,000.

The diffusion constant estimated from the spread of the sedimenting boundary was  $4.0 \times 10^{-7}$  cm.<sup>2</sup> per sec., it being fully recognized that values obtained in this way from sedimentation curves are only approximations. If the particles are spherical, this indicates that the true micellar weight may be as high as 400,000 and the density as low as 1.10.

(5) Aqueous 5 per cent solutions of sodium desoxycholate and a crystalline preparation of bile salts (mostly sodium glycocholate) were also studied. It was of considerable interest to test these substances since they possess a hydrophobic nucleus similar to that of digitonin, but differ in that they are electrolytes. The refractive index curves of these substances showed no detectable quantities of micelles of more than a few thousand in molecular weight. The type of curve obtained (Fig. 2) was characteristic of particles of relatively low molecular weight, there being no boundary but only a decrease of concentration in the upper part of the solution and some increase in the lower section. The shape of the curve changed little even on prolonged centrifugation. A similar result was obtained with solutions of sodium dodecyl sulphate.

(6) The random spread of particle size exhibited by the more familiar colloidal aggregates such as gold sols has usually been accepted as a distinguishing characteristic of colloids in general, in contrast to the well-defined molecular sizes of pure protein preparations. The homogeneous micelles of digitonin provide an interesting example of a "colloidal" particle, which is not consistent with this viewpoint, and it is quite possible that other substances of mixed hydrophobic and hydrophylic nature which act as detergents may also show this property. Large micelles are not

likely to be found among the electrolytes; this is indicated by the three studied by us, and also by those studied by McBain and Laing-McBain.<sup>5</sup>

Detergents have long been used for the dispersal of various types of substances of biological interest, particularly the proteins. It is important to emphasize that the detergent may not only affect the proteins studied but that some of its properties such as the molecular size may fall within that range usually considered to be characteristic of proteins alone.

*Summary.*—Ultracentrifugal observations using a direct reading refractive index method have been made on aqueous solutions of digitonin. Practically all of the digitonin exists in the form of micelles of homogeneous size, with an average sedimentation constant of  $5.88 \times 10^{-13}$  cm. per dyne per sec. The micellar weight is likely to lie within the range of 75,000 to 400,000, as contrasted with a molecular weight of 1228.

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## A SET OF POSTULATES FOR BOLYAI-LOBATCHEVSKY GEOMETRY

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1. *Introduction.*—In some recent papers<sup>1</sup> Menger proved that all concepts of the Bolyai-Lobatchevsky geometry can be defined in terms of the operations "joining" and "intersecting," basic to his algebra of projective and affine geometry, as well as to G. Birkhoff's lattice theory. It follows that a complete foundation of non-Euclidean geometry can be given in terms of these two concepts, e.g., by substituting into the ordinary postulates Menger's definitions of the concepts "between," "parallel," "congruent," etc., in terms of joining and intersecting. Since postulates obtained in this way would be very cumbersome, there arose the problem of establishing some simple direct postulates concerning the two operations



from which the non-Euclidean geometry could be developed. In what follows we give a list of eight postulates from which the whole theories of order and parallelism in non-Euclidean geometry can be derived.

We restrict ourselves to the case of the plane and use the concepts of "point," "line" and "lies on," which as is well known can be derived from the operations of joining and intersecting. Our present definitions of betweenness and parallelism differ from those originally given by Menger in that they avoid the use of "maximal triangles," and are stated directly in terms of the primitive notions.

2. *The Postulates.*—The following postulates will be assumed.

I. If  $A$  and  $B$  are any two distinct points, there exists exactly one line  $m$  such that both  $A$  and  $B$  lie on  $m$ .

II. Each line contains at least five distinct points.

III. There exist at least three non-collinear points.

IV. If  $a, b$  are any two distinct intersecting lines, and  $P$  is a point not on  $a$  or  $b$ , then there exists at least one line through  $P$  which intersects  $a$ , but not  $b$ .

V. If  $a, b$  are any two non-intersecting lines, and  $P$  is any point not on  $a$  or  $b$ , then there exists a line through  $P$  which intersects neither  $a$  nor  $b$ .

VI. If  $A, B, C$  are distinct collinear points, and if there exist lines  $a$  and  $a'$  through  $A$ ,  $b$  and  $b'$  through  $B$ ,  $c$  and  $c'$  through  $C$ , such that

1)  $b$  and  $c$  intersect, but neither meets  $a$ ,

2)  $a'$  and  $b'$  intersect, but neither meets  $c'$ ,

then each line through  $B$  meets at least one line of every pair of intersecting lines which pass through  $A$  and  $C$ , respectively.

VII. If  $a, b, c$  are three mutually non-intersecting lines, and if there exists a line meeting both  $a$  and  $b$ , but not  $c$ , and also a line meeting  $b$  and  $c$ , but not  $a$ , then through any point of  $a$ , there exists a line meeting  $b$  but not  $c$ .

VIII. If  $a$  is a given line, then through any point not on  $a$  there exist at least two distinct lines which do not intersect  $a$ .

Postulate VIII is the only one which is not satisfied equally in the Euclidean plane, VI and VII being vacuously satisfied there. All but IV and VIII are true even in the projective plane.

3. *The Theory of Order.*—We say of three distinct points  $A, B, C$  that  $B$  lies *between* the two other points  $A$  and  $C$ , if every line through  $B$  intersects at least one line of each pair of intersecting lines which pass through  $A$  and  $C$ , respectively. We show that any three points satisfying this definition must be collinear, and that the triadic relation so defined satisfies the conditions for a betweenness relation given by Huntington and Kline.<sup>2</sup> Moreover, we derive the statement known as the axiom of Pasch: If a line meets one side of a triangle in an interior point, then it meets exactly one of the other two sides in an interior point, or passes through the op-



posite vertex. Finally, the plane is shown to be convex and externally convex, i.e., to each pair of points  $A, C$ , there exist two points  $B, D$  such that  $B$  lies between  $A$  and  $C$ , and  $C$  lies between  $A$  and  $D$ . These results imply that each line separates the plane into two parts.

4. *The Theory of Parallelism.*—Two non-intersecting lines  $a$  and  $b$  are said to be *parallel* if there exists a point  $P$  such that through  $P$  there is at most one line which meets neither  $a$  nor  $b$ . This definition is proved to be independent of  $P$  in the sense that each point lying between  $a$  and  $b$  has the same property as  $P$ . Here we say that the point  $Q$  lies between the non-intersecting lines  $m$  and  $n$  if there exists a line through  $Q$  which intersects  $m$  and  $n$  in points  $M$  and  $N$ , respectively, such that  $Q$  lies between  $M$  and  $N$ . The relation of parallelism is clearly symmetric, and has the property that if  $a$  is a parallel to  $b$  through a point  $Q$ , and if  $R$  is any other point of  $a$ , then  $a$  is also a parallel to  $b$  through  $R$ .

Let  $c$  be a line which intersects two parallels  $a, b$  in the points  $A, B$ , respectively, and  $P$  be a point of  $a$ , distinct from  $A$ . We say that  $a$  and  $b$  are parallel on the side of  $c$  on which  $P$  lies, if there exists a line through  $P$  intersecting  $c$  in a point between  $A$  and  $B$ , but not intersecting  $b$ . Here again the definition is shown to be independent of the point  $P$ , for we prove that any point of  $a$  or  $b$  which is on the side of  $c$  on which  $P$  lies may replace  $P$  in the definition. Of course, for a line  $c$  the lines  $a$  and  $b$  may be parallel on the side of  $c$  on which  $P$  lies, whereas for another line  $d$ , they might be parallel on the side of  $d$  opposite to that on which  $P$  lies.

If  $a$  and  $b$  are parallel, and  $b$  and  $c$  are parallel, and  $t$  is a line intersecting each of the lines  $a, b, c$ , then  $a$  and  $c$  are said to be parallel to the line  $b$  on the same side of the transversal  $t$  if there exists a point  $P$  on  $b$  such that both  $a$  and  $b$ , and  $b$  and  $c$  are parallel on that side of  $t$  on which  $P$  lies.

Using these concepts, we prove the classical assumption that the relation of parallelism is transitive in a certain sense: If  $a$  and  $c$  are two lines which are parallel to  $b$  on the same side of a transversal, then  $a$  is parallel to  $c$ . Further, if  $c, d$  are two parallels to a line  $m$  through a point  $P$ , then  $c$  and  $d$  are parallel to  $m$  on opposite sides of any line through  $P$  meeting  $m$ , and hence there exist at most two parallels to any given line through a given point.

For the full development of the above results, see the author's papers in the *Reports of a Mathematical Colloquium*, Issue 1, pp. 45–48, Issues 2 and 3 in press.

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<sup>2</sup> *Trans. Amer. Math. Soc.* 18, 301–325 (1917).

# A GENERAL THEORY OF SPECTRA. I

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The mathematical theory of spectra deals with the characteristic value problem (Eigenwertproblem) for linear operators, and provides a general unifying treatment for typical instances of the problem occurring in applied mathematics. Of recent years the tendency to emphasize the algebraic aspects of the spectral theory has become more and more pronounced. This tendency is quite as evident in the applications as in the purely mathematical developments, being a characteristic feature of the quantum theory and of the Heaviside calculus. In the present note we sketch further steps in the direction of a thorough "algebraization" of the spectral theory: we shall show that without the mediation of any theory of integration it is possible to define general functions of operators and to elaborate their calculus.

We consider a system  $R$  of elements  $a, b, c, \dots$ , which for purposes of illustration may be interpreted as operators, together with a special subsystem  $P$  of elements called "positive." We require that

(1) in terms of addition and multiplication,  $R$  is a commutative, associative ring with unit  $e$ ;

(2) for each natural number  $n$  the equation  $nx = e$  has a solution in  $R$ ;

(3) sums and products of positive elements are positive, but  $a$  and  $-a$  are both positive only in case  $a = 0$ ;

(4) the square of any element is positive;

(5) if  $a$  is given, there exists a natural number  $n$  such that  $ne + a$  is positive;

(6) if  $e + na$  is positive for every natural number  $n$ , then  $a$  is positive.

These properties lead at once to a number of simple results: the elements of  $R$  admit multiplication by the rational numbers; each element  $a$  can be assigned a real number  $\|a\|$  as its norm; the elements of  $R$  can be partially ordered by defining  $a < b$  if  $b - a$  is positive and not equal to 0. We require further that

(7) with the distance  $\|a - b\|$  the system  $R$  is a complete metric space.

Actually, of course, we should expect to be able to achieve the satisfaction of (7) by a completion process of familiar type, adjoining new elements to  $R$  so as to obtain an enlarged system  $R^*$  with an enlarged class  $P^*$  of positive elements enjoying all the properties (1)–(7). This proves to be the case. What we can now establish is this: *The system  $R$  described above is algebraically isomorphic to the ring of ALL continuous real functions on*

a certain bicomact Hausdorff space  $S(R)$ , the positive elements of  $R$  corresponding precisely to the non-negative functions; and  $S(R)$  is uniquely determined up to topological equivalences.<sup>1</sup> Since the continuous real functions on any bicomact Hausdorff space constitute a system with properties (1)–(7), it follows that these properties characterize in algebraic and ordinal terms such classes of real functions.<sup>1</sup> To prove these results, one combines general principles of algebra and topology with information concerning the existence and properties of square roots in the given system  $R$ . Without going into detail, it is of interest to observe that the determination of the positive square root of a positive element  $a \leq e$  is most conveniently effected through the continued fraction algorithm set up by converting the equation  $x^2 = a$  into the equivalent form  $(e + x)x = a + x$  and writing the latter, at first in a purely formal sense, as  $x = e - (e - a)/(e + x)$ .

It is now evident that, if  $a$  is any element of  $R$  and  $F(\lambda)$  is any continuous real function defined for all real  $\lambda$ , then  $F(a)$  can be uniquely interpreted as an element of  $R$ : for, if  $f$  is that continuous function on  $S(R)$  which represents  $a$  in the isomorphism described above,<sup>2</sup> then  $F(f)$  is also a continuous function on  $S(R)$  and thus represents a certain element of  $R$  which may appropriately be denoted as  $F(a)$ . The development of a complete operational calculus of such functions of elements in  $R$  can therefore proceed in an obvious way. If  $F$  is not continuous,  $F(f)$  cannot in general be correlated with an element of  $R$  but still has significance as a function on  $S(R)$ . Consequently,  $R$  can be so enlarged that  $F(a)$  has meaning in the extended system even when  $F$  is not continuous: for example, in dealing with bounded functions  $F$ , we may use as the extended system the class of all bounded real functions on  $S(R)$ , for which properties (1)–(7) are readily verified. If it is desired to treat non-bounded functions  $F$ , a similar procedure is possible but the extension of  $R$  employed cannot in general have properties (5) and (7).

In many cases, however, no enlargement of  $R$  is necessary in order to set up an operational calculus in terms of a wide class of discontinuous functions  $F$ . Let us require that, instead of the property (7),  $R$  have the property

(7') if  $\{a_n\}$  is a sequence of positive elements with  $a_n \geq a_{n+1}$ , then it has a greatest lower bound.

Property (7') implies property (7); moreover, it is equivalent to the following property of the associated space  $S(R)$ : every bounded Baire function on  $S(R)$  differs only on a set of first category from a continuous function uniquely associated with it. In the proof of this equivalence, we establish further that both property (7') and the property of  $S(R)$  indicated above are equivalent to the following property:  $S(R)$  is the representative Boolean space<sup>3</sup> for a completely additive Boolean algebra, which can be realized by means of the idempotent elements of  $R$ . Returning to the interpretation

of  $F(a)$ , we see at once that, when  $R$  has property (7') and when  $F$  is a bounded Baire function,  $F(f)$  is a bounded Baire function on  $S(R)$  determining a unique continuous function and a unique corresponding element of  $R$ , which may appropriately be denoted as  $F(a)$ . We thus obtain a complete operational calculus with bounded Baire functions  $F$ , applicable entirely *within* the system  $R$ . It is not difficult to see that (7') is essentially a necessary as well as a sufficient condition for the constructibility of such a calculus.

The general concepts outlined above can be illustrated or applied in a variety of ways. Examples of systems  $R$  which lead to unexpected interpretations and results are: the class of all bounded continuous real functions on an arbitrary topological space;<sup>1</sup> the class of all bounded Lebesgue measurable functions on a general domain, a function being considered positive if it is negative only on a set of measure zero. It is verifiable by quite elementary considerations that any abelian ring of bounded self-adjoint operators in Hilbert space is a system  $R$  which possesses property (7') in a much strengthened form. The present theory therefore includes as a special case the simultaneous spectral analysis of any number of mutually permutable bounded self-adjoint operators together with the development of their operational calculus. In order to treat non-bounded operators, it suffices to use one of the available methods for reduction of the non-bounded to the bounded case. Interpreting this instance of a system  $R$  in physical terms, we have a treatment of any system of real, simultaneously observable physical quantities as envisaged in the quantum theory.<sup>4</sup> The formal systems described by Steen<sup>5</sup> as a basis for an abstract analogue of the theory of self-adjoint operators can be brought into intimate relation with the present theory, as one would expect; but it should be observed that Steen's considerations remain on a more formal level than ours, in the sense that they do not serve to identify the systems considered. There exist similar connections between the present note and a theory initiated by von Neumann;<sup>6</sup> but our results apply only to associative subsystems of von Neumann's non-associative algebras. In the present outline, we have had occasion to make certain references to the theory of Boolean algebras. That these references are neither accidental nor forced appears from the fact that the general theory of such algebras as we have developed it elsewhere<sup>3</sup> is a special instance of the present theory: if one considers the formal linear forms with rational coefficients built from an abstract Boolean ring  $A$  and treats them in an appropriate way, as though they represented "step-functions," one obtains a system  $R$  which can be completed so as to have properties (1)–(7); the resulting bicomact Hausdorff space is precisely the Boolean space attached to  $A$ . Actually it is simpler to develop the theory of Boolean algebras independently, since many aspects of the general theory described here either become trivial or can be circumvented

in a direct treatment of this special case. Finally, we observe that the concepts of the present note illuminate (and even introduce certain technical simplifications into) recent work of Bochner on finitely additive integrals<sup>7</sup> and of Bochner and Wecken on almost periodic functions.<sup>8</sup>

In a second note we shall discuss the parallel between the present theory and certain results of the theory of linear lattices. In particular we shall show that the general principles developed here carry over to yield an integration-free treatment of Riesz's operational calculus in a linear lattice.<sup>9</sup>

<sup>1</sup> For discussions of the properties of continuous functions involved in this context, see M. H. Stone, *Trans. Am. Math. Soc.*, **41**, 375-481 (1937), especially Chapter III; and E. Čech, *Ann. Math. (2)* **38**, 823-844 (1937).

<sup>2</sup> The range of this function  $f$  is the spectrum of  $a$ .

<sup>3</sup> See M. H. Stone, *loc. cit.*,<sup>1</sup> especially Chapter I, and the earlier work cited there.

<sup>4</sup> P. A. M. Dirac, *The Principles of Quantum Mechanics*, 1931.

<sup>5</sup> S. W. P. Steen, *Proc. Lond. Math. Soc. (2)* **41** 361-392 (1936); **43**, 529-543 (1937); **44**, 398-411 (1938); **45**, 562-578 (1939). The last two papers are concerned with non-commutative systems closely related to those cited in ref. 6.

<sup>6</sup> J. von Neumann, *Matematicheskii Sbornik*, **1** (43) 415-484 (1936).

<sup>7</sup> S. Bochner, *Ann. Math. (2)* **40**, 769-799 (1939).

<sup>8</sup> S. Bochner, *loc. cit.*;<sup>7</sup> F. J. Wecken, *Math. Zeit.*, **45**, 377-404 (1939).

<sup>9</sup> F. Riesz, *Ann. Math. (2)* **41**, 174-206 (1940).

## THE GROUPS WHICH CONTAIN EXACTLY FOURTEEN PROPER SUBGROUPS

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Every abelian group whose order is the product of four distinct prime numbers is cyclic and contains exactly fourteen proper subgroups. If the order of an abelian group is divisible by three and only three distinct prime numbers and contains exactly fourteen proper subgroups it is cyclic and this order is of the form  $p_1 p_2 p_3^2$ ,  $p_1$ ,  $p_2$  and  $p_3$  being any three distinct prime numbers. Moreover, every such group contains exactly fourteen proper subgroups. If the order of a cyclic group has only two distinct prime factors and this group contains exactly fourteen proper subgroups this order is of one of the following two forms  $p_1 p_2^7$ ,  $p_1^3 p_2^3$ ,  $p_1$  and  $p_2$  being distinct prime numbers, and every such group contains exactly fourteen proper subgroups.

If an abelian group whose order has two distinct prime factors is non-cyclic it is the direct product of its two Sylow subgroups and one and only one of these Sylow subgroups is non-cyclic if the group contains exactly



fourteen proper subgroups. This non-cyclic group may be the group of order 8 and of type 2, 1 while the other Sylow subgroup is an arbitrary group of odd prime order, or it may be the non-cyclic group of order 25 while the other Sylow group is an arbitrary group of prime order with the exception of the group of order 5. Hence there are two and only two such non-cyclic abelian groups, whose orders are of the form  $8p$  and  $25p$ , respectively, where  $p$  is any prime number except one in each case. If a prime power group is abelian and contains exactly fourteen proper subgroups it is of order 8 and of type  $1^3$  if it has as many as three invariants, of order 169 and of type  $1^3$  if it has two invariants, and of order  $p^{16}$  if it is cyclic. Hence *there are nine abelian groups which separately contain exactly fourteen proper subgroups*. Seven of these are infinite systems of groups while two are individual groups.

If a non-abelian prime power group contains exactly fourteen proper subgroups this prime number cannot exceed 3 since a non-abelian group whose order is a power of an odd prime number  $p$  contains a non-cyclic invariant subgroup of order  $p^2$ . If this prime number were 3 then  $G$  would contain an invariant subgroup of order 27 which would involve exactly 8 subgroups but it would then also involve 13 subgroups of order 3 since it could not contain a subgroup of order 81 which involves exactly 12 subgroups. Hence the order of  $G$  must be a power of 2 if  $G$  is a non-abelian prime power group and contains exactly fourteen proper subgroups. It would involve an invariant cyclic subgroup of order 8 since the groups of order 16 which do not involve such an invariant subgroup involve at least 13 proper subgroups. As this is impossible it has been proved that no non-abelian prime power group contains exactly fourteen proper subgroups. Before considering the non-abelian groups which contain exactly fourteen proper subgroups and have separately an order which is divisible by two distinct prime numbers it may be desirable to consider a general theorem which proves the existence of several of these groups as special cases. Consider the dihedral group of order  $2p$ ,  $p$  being an odd prime number, and establish a  $p, 2^m - 1$  isomorphism between it and the cyclic group order  $2^m$ . The resulting group is of order  $p2^m$  and it contains  $p + 1 + 2(m - 1)$  proper subgroups since it contains a set of  $p$  conjugate cyclic subgroups of order  $2^m$ . It is of an arbitrary even order beginning with the order  $p + 1$ . Moreover, when the values of  $p$  and  $m$  are given, there is one and only one such group. Hence there results the following THEOREM: *When  $p$  is an arbitrary odd prime number and  $m$  is an arbitrary positive integer there is a non-abelian group of order  $p2^m$  which contains exactly  $p + 1 + 2(m - 1)$  proper subgroups.*

If we let  $p + 1 + 2(m - 1) = 14$  there results one group from this theorem for each of the prime numbers 3, 5, 7, 11, 13. Hence there result therefrom five non-abelian groups which separately contain exactly



fourteen proper subgroups and are of the following orders: 192, 160, 112, 44, 26. It should be noted that this theorem yields at least one non-abelian group which has an arbitrary even number of proper subgroups greater than 2. When the even number is 4 this theorem yields the non-cyclic group of order 6. There is in this case also the quaternion group which contains exactly four proper subgroups but does not come under this theorem. Since the cyclic group of order  $p^m$ ,  $p$  being an arbitrary prime number, contains exactly  $m - 1$  proper subgroups there is at least one group which contains an arbitrary given number of subgroups, and from the theorem of the preceding paragraph it results that there are at least two groups which contain exactly any given even number of proper subgroups whenever this even number exceeds 2. The number of these subgroups exceeds the number of odd prime numbers which are less than this even number.

When the non-abelian group  $G$  contains exactly fourteen proper subgroups and has an order which is divisible by two and only two distinct prime numbers the larger of these prime numbers cannot exceed 13 and when it is 13,  $G$  either comes under the theorem noted above or it is the semi-metacyclic group of order 39. When the larger of these prime numbers is 11 there is again one group besides the one which comes under the given theorem, viz., the group obtained by establishing a 11, 5 isomorphism between the semi-metacyclic group of order 55 and the cyclic group of order 25. When the larger of the two prime factors of the order of  $G$  is 7 there is again one group besides the one which comes under the given theorem, viz., the one obtained by establishing a 7, 27 isomorphism between the semi-metacyclic group of order 21 and the cyclic group of order 81.

When the larger of the two prime factors of the order of  $G$  is 5 the additional group besides the one resulting from the given theorem is obtained by establishing a 5, 2 isomorphism between the dicyclic group of order 20 and the cyclic group of order 8. It remains to consider the possible groups when the larger of the prime factors which divide the order of  $G$  is 3. The number of the Sylow subgroups whose orders are powers of 3 could then not exceed 4. In fact, it will be seen that this number could not be 4 since  $G$  would then be isomorphic with the tetrahedral group and hence it would contain an invariant subgroup of index 3 which would itself contain three invariant subgroups of index 2. This subgroup of index 3 could not be of order 8 since the non-twelve group of order 24 contains exactly thirteen proper subgroups. It could not be of order 12 since the corresponding group of order 36 also contains exactly thirteen subgroups. As it could not be of any larger order it results that the Sylow-subgroup whose order is a power of 3 is invariant under  $G$  when  $G$  involves exactly fourteen proper subgroups.

The Sylow-subgroups of  $G$  whose order is a power of 2 cannot be invariant since  $G$  is not the direct product of its Sylow-subgroups. If the number of these Sylow-subgroups is 9 then  $G$  is the dihedral group of order 18. It remains to consider the case when  $G$  contains three Sylow subgroups whose order is a power of 2 and an invariant subgroup whose order is a power of 3. This invariant subgroup could not have an order which exceeds 3 and hence  $G$  is the dihedral group of order 12 since the case when it is a 3, 32 isomorphism between the non-cyclic group of order 6 and the cyclic group of order 64 comes under the theorem noted above. It may be added that the dihedral group of order 12 is the direct product of the group of order 2 and the non-cyclic group of order 6. Combining these results it follows that *there are eleven non-abelian groups which separately contain exactly fourteen proper subgroups and whose orders are divisible by two distinct prime numbers*. These are all individual groups in the sense that none of them represents an infinite system of groups.

A group which contains no more than fourteen proper subgroups is clearly solvable and hence its order cannot be divisible by as many as four distinct prime numbers when it is non-abelian. If its order is divisible by exactly three distinct prime numbers it may be the direct product of the dihedral group of order 10 and a group of prime order when this order is neither 2 nor 5. It may also be the direct product of the dicyclic group of order 12 and the group of order  $p$ , where  $p$  is any prime number which exceeds 3. No one of these three prime numbers which divide the order of  $G$  can exceed 5 unless  $G$  is the direct product of two groups of which one is of this prime order. If the order of  $G$  is divisible by 5 and  $G$  is not the direct product of a group of order 5 and some other group then  $G$  must contain the dihedral group of order 10 and be the direct product noted above. If  $G$  is the direct product of a group of prime order and a non-abelian group whose order is not divisible by as large a prime order as 5 then  $G$  is the direct product noted above. Hence there results the following THEOREM: *The total number of groups which separately contain exactly fourteen proper subgroups is twenty-two.*

## EQUILONG SYMMETRY WITH RESPECT TO ANY CURVE

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1. *Introduction.*—In this paper, we shall define a certain geometric operation, called *equilong symmetry*. This may be considered as a kind of dual of conformal symmetry, which is identical with Schwarzian reflection in an arbitrary analytic curve. Equilong symmetry is defined for an arbitrary base curve in line geometry. The conformal theory is limited to space of two dimensions, but the equilong theory is readily extended to three or more dimensions.

When the base curve is a circle, conformal symmetry becomes Moebius inversion. On the other hand, when the base curve is a circle, the analogous equilong symmetry does *not* become Laguerre inversion, but a new correspondence, which we call *K inversion*.

Conformal transformations are correspondences between the  $\infty^2$  points of the plane which preserve or reverse the angle between the two directions of any two curves at their common point of intersection. Equilong transformations are correspondences between the  $\infty^2$  lines of the plane which preserve or reverse the distance between the two points of contact of any two curves along their common tangent line. Conformal transformations are defined by monogenic functions of the complex variable  $x \pm iy$  where  $i^2 = -1$  and  $(x, y)$  are the cartesian coördinates of a point; whereas equilong transformations are given by monogenic functions of the *dual* variable  $u \pm jv$  where  $j^2 = 0$  and  $(u, v)$  are the hessian or equilong coördinates of a line.

For any given curve  $C$ , there is a unique reverse conformal transformation  $S$ , which leaves fixed the points of  $C$ ; and also *there is a unique reverse equilong transformation  $S^*$ , which leaves fixed the tangent lines of  $C$* . The correspondence  $S$  is called conformal symmetry (Schwarzian reflection), and the correspondence  $S^*$  is said to be equilong symmetry. The latter is our new operation which, in the present paper, we shall consider briefly with respect to an arbitrary curve, and then rather extensively with respect to a circle.<sup>1</sup>

In studying the line geometry of the plane, a lineal element  $E$  is usually defined by the hessian coördinates  $(u, v, w)$ . But we shall find it more convenient to define a lineal element  $E$  by the *equilong coördinates*  $(x, y, p = dy/dx)$ . The hessian and equilong coördinates are connected by the relations

$$x = \tan u/2, y = \frac{1}{2}v \sec^2 u/2, p = w + v \tan u/2. \quad (1)$$

The inverse of this correspondence is

$$u = 2 \arctan x, v = \frac{2y}{1+x^2}, w = p - \frac{2xy}{1+x^2}. \quad (2)$$

We wish to emphasize here that the equilong coördinates are *not* the cartesian coördinates of an element  $E$  although the notation is the same.

2. *The Group  $G'_{sym}$  of Equilong Symmetries and Translations.*—The equilong symmetry  $S^*$  with respect to the curve  $C: y = f(x)$  is defined as the unique reverse equilong correspondence which leaves fixed the tangent lines of  $C$ . By imposing this condition upon the set of reverse equilong transformations, the following proposition is found to be true.

**THEOREM 1.** *The unique equilong symmetry  $S^*$  with respect to the curve  $C: y = f(x)$  is given by*

$$X = x, Y = -y + 2f(x). \quad (3)$$

By means of the direct equilong correspondence  $X = x, Y = y - f(x)$ , it may be shown that any equilong symmetry is the transform of the ordinary point symmetry through the origin  $X = x, Y = -y$  under the group  $G$  of direct equilong transformations.

When the curve  $C$  is given, the real construction of the conformal symmetry  $S$  is extremely difficult and has been accomplished in our previous work by successive approximations, using the normals to  $C$  and the curvature  $\gamma$  and the higher derivatives of  $\gamma$  for all orders with respect to the arc length  $s$  of  $C$ .<sup>2</sup> On the other hand, it is found by means of the preceding equations that when  $C$  is given, the direct construction of  $S^*$  is easy. Let  $l$  be any (oriented) line in the plane. Construct the tangent line  $t$  of the curve  $C$  which is parallel to  $l$ . (Two oriented lines are parallel if their point of intersection is at infinity and also if they possess the same orientation.) The correspondent  $L$  of  $l$  under  $S^*$  is a line parallel to both  $l$  and  $t$  such that  $t$  is the bisector of the perpendicular distance between  $l$  and  $L$ . Thus we have

**THEOREM 2.** *The construction of the equilong symmetry  $S^*$  with respect to a curve  $C$  is accomplished by means of ordinary symmetry in the respective parallel tangent lines of  $C$ .*

The product of two equilong symmetries  $S_2^* S_1^*$  is *not* an equilong symmetry. We shall call any such transformation an *equilong translation*.

**THEOREM 3.** *Any equilong translation is given by*

$$X = x, Y = y + g(x). \quad (4)$$

By means of the direct equilong transformation  $X = \int (x/g) dx, Y = (x/g)y$ , we find that any nonidentical equilong translation is the transform

of the ordinary translation  $X = x$ ,  $Y = y + x$  under the group of equilong transformations.

The group generated by all conformal symmetries has already been discussed in another of our papers.<sup>3</sup> The main result of this preceding paper is that *any conformal transformation is the product of a finite number (not exceeding four) of conformal symmetries followed by an homothetic transformation*. Concerning the group generated by equilong symmetries, we discover the following conclusion.

**THEOREM 4.** *The group generated by equilong symmetries is a certain mixed group  $G'_{\text{sym}}$  consisting of equilong symmetries and translations, expressed as follows*

$$X = x, Y = \mp y + \psi(x). \quad (5)$$

In the dual variable  $z = x + jy$ ,  $j^2 = 0$ , this mixed group  $G'_{\text{sym}}$  may be written as

$$Z = \bar{z} + j\psi(\bar{z}), Z = z + j\psi(z), \quad (6)$$

where in either case the function  $\psi$  is a power series with real coefficients and  $\bar{z} = x - jy$  is the conjugate of  $z = x + jy$ . The first is an equilong symmetry and the second is an equilong translation.

Any transformation of this group  $G'_{\text{sym}}$  may be defined as a line correspondence which carries any line into one parallel to itself, and which preserves or reverses the distance between any two parallel lines. Of course,  $G'_{\text{sym}}$  contains the group of equilong translations as a subgroup.

**3. The Group  $G'_3$  Generated by  $K$  Inversions.**—In this section, we shall specialize our results of the preceding section to the case where the fixed curve is a circle. First, let us note that in equilong coördinates any circle is represented by a vertical parabola

$$y = ax^2 + bx + c. \quad (7)$$

We shall use  $C(a, b, c)$  as the coördinates of a circle.

Recalling that  $K$  inversion is defined as equilong symmetry with respect to the circle  $C(a, b, c)$ , we find that it is represented, in accordance with (3), by

$$X = x, Y = -y + 2ax^2 + 2bx + 2c. \quad (8)$$

Any such transformation is the transform of ordinary point symmetry under a dilatation. Obviously

*Any  $K$  inversion carries any circle into a circle.*

The product  $S = S_{2n-1}S_{2n-2}\dots S_2S_1$  of an odd number of  $K$  inversions with respect to  $2n-1$  circles is also a  $K$  inversion. To determine the circle  $C$  of  $S$ , we proceed as follows. Let  $D_1, D_2, \dots, D_{2n-1}$  denote the centers, and  $S_1, S_2, \dots, S_{2n-1}$  denote the end-points of the  $2n-1$  parallel



radii of the circles of the  $2n - 1$  equiangular symmetries. Let  $D_{2n}$  be the point such that the vector sum of the alternate sides of the polygon whose vertices are  $D_1, D_2, \dots, D_{2n-1}, D_{2n}$  is zero. Similarly let  $S_{2n}$  be the point such that the vector sum of the alternate sides of the polygon whose vertices are  $S_1, S_2, \dots, S_{2n-1}, S_{2n}$  is zero. The circle whose center is  $D_{2n}$  and which passes through the point  $S_{2n}$  is the required circle  $C$  of the  $K$  inversion  $S$ .

The product of two  $K$  inversions with respect to two circles is *not* a  $K$  inversion. We shall call such a correspondence a  $K$  translation. Obviously any such transformation is of the form

$$X = x, Y = y + hx^2 + kx + l. \quad (9)$$

From these equations, we find that any equiangular translation is simply the product of an ordinary translation by a dilatation. By (9), it is immediately seen that the set of all translations forms a three-parameter group  $G_3$ .

The group  $G_3$  of  $K$  translations is a subgroup of the mixed three-parameter group  $G'_3$  generated by all  $K$  inversions. This mixed group  $G'_3$  is given in equiangular coordinates by

$$X = x, Y = y + ax^2 + bx + c. \quad (10)$$

In the dual variable, the mixed group  $G'_3$  may be written as

$$Z = \frac{(a + ja')\bar{z} + jb}{jc\bar{z} + (a + jd)}, Z = \frac{(a + ja')z + jb}{jcz + (a + jd)}, \quad (11)$$

where  $a \neq 0$ ,  $a', b, c, d$  are real numbers. The first is a  $K$  inversion and the second is a  $K$  translation.

A Laguerre inversion may be written in the dual variable as

$$Z = \frac{\alpha\bar{z} + b}{c\bar{z} - \bar{\alpha}}, \quad (12)$$

where  $b, c$  are real numbers and  $\bar{\alpha}$  is the conjugate of the dual number  $\alpha$ . This has the same form as a Moebius inversion written in the ordinary complex variable notation. The group generated by Laguerre inversions is the mixed six-parameter Laguerre group  $G'_6$ .

$$Z = \frac{\alpha\bar{z} + \beta}{\gamma\bar{z} + \delta}, Z = \frac{\alpha z + \beta}{\gamma z + \delta}. \quad (13)$$

By comparing (11), (12) and (13), we find that a Laguerre inversion can *never* be identical with a  $K$  inversion, and conversely. Nevertheless we obtain the following result:

**THEOREM 5.** *Any  $K$  inversion is the product of three Laguerre inversions and any  $K$  translation is the product of two Laguerre inversions.*

It is significant to note that the  $K$  inversions do not generate the entire



Laguerre group  $G'_\delta$  of circle transformations but only a certain subgroup  $G'_\delta$  of it. These remarks show that the equilong theory is entirely different in this respect from the conformal theory, where conformal symmetry with respect to a circle is Moebius inversion and hence generates the entire mixed six-parameter Moebius group  $G'_\delta$ .

4. *Other Distinctions between the Two Theories.* In the case of conformal (Schwarzian) symmetry  $S$ , it is essential that the base curve  $C$  shall be analytic. (This is used in the theory of analytic prolongation.) But in the case of our new equilong symmetry  $S^*$ , the base curve  $C$  may be any curve with continuously turning tangent (and even further generalization is possible).

If we take a *horn angle* and *bisect* it in the *conformal manner*, the intrinsic quantities curvature and the first and second derivatives with respect to arc length take exactly the average values for the middle curve; this is not true of the third and higher derivatives.<sup>2</sup>

If we bisect the horn angle in the *equilong manner*, then the appropriate intrinsic quantities are radius of curvature and derivatives with respect to inclination; and we find that these take *average values for all orders*.<sup>1</sup> Thus there are many analogies and many distinctions.

<sup>1</sup> Kasner, "Conformal and Equilong Symmetries," *Science*, 83, 480 (1936).

<sup>2</sup> Kasner, "Geometry of Conformal Symmetry (Schwarzian Reflection), *Ann. Math.*, 38, 873-879 (1937); Comenetz, "Conformal Geometry on a Surface," *Ibid.*, 39, 863-871 (1938).

<sup>3</sup> Kasner, "Infinite Groups Generated by Conformal Transformations of Period Two (Involutions and Symmetries)," *Am. Jour. Math.*, 38, 177-184 (1916).

## PARTIALLY ORDERED SETS AND TOPOLOGY

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1. *Separating Systems.*—In the following we obtain the Brouwer reduction theorem and the Borel covering theorems by applying theorems about partially ordered sets to systems of closed subsets of a topological space. Moreover, we formulate conditions on a partially ordered set  $P$  necessary and sufficient to guarantee the existence of a topological space having a basis of closed sets isomorphic to  $P$ . In both problems we use the notion of separating system.

Let  $\mathfrak{L}$  be a collection of lower sections<sup>1</sup> of  $P$ . We call  $\mathfrak{L}$  a separating system (strong separating system) if for each  $x$  and  $y$  of  $P$  such that  $x < y$  ( $y$  not  $\leq x$ ) there is a lower section  $L$  in the collection  $\mathfrak{L}$  such that  $x$  belongs

to  $L$ , and  $y$  does not belong to  $L$ . In an analogous way a collection of upper sections may be defined to be a separating system.

Many properties of partially ordered sets are consequences of the existence of a separating system whose power is sufficiently small.

2. *Extremal Sequences and the Reduction Theorem.*—Let  $P$  be a partially ordered set and  $\mathfrak{A}$  a separating system of power  $M$ . For each element  $x$  of  $P$  either there is an element  $x'$  of  $P$  such that  $x' \leq x$  and no element of  $P$  is  $<$  than  $x'$ , or there is a monotonically decreasing sequence  $x_1 = x, x_2, \dots, x_\alpha, \dots$  defined for all  $\alpha < \kappa$  where  $\kappa$  is an ordinal number not exceeding the first ordinal of power  $M$ . Such a sequence is called a minimal sequence starting with  $x$ .

We shall call  $P$  *lower inductive of power  $M$*  if, whenever  $x_1, x_2, \dots$  is a monotonically decreasing sequence of elements of  $P$  of power not exceeding  $M$ , there exists an element  $x$  of  $P$  such that  $x \leq x_\alpha$  for each  $x_\alpha$  of the sequence. *If  $P$  is lower inductive of power  $M$ , and has a separating system of power at most  $M$ , then for each element  $x$  of  $P$  there exists at least one element  $x' \leq x$  such that no other element of  $P$  is  $< x'$ .*

One consequence of this result is the Brouwer reduction theorem, since for a topological space having a denumerable basis, any partially ordered set of closed subsets has a denumerable separating system,<sup>2</sup> and the assumption that such a partially ordered set  $P$  is lower inductive of denumerable power implies that each of the sets belonging to  $P$  contains a smallest set belonging to  $P$ .

3. *The Zero Element in Subsets of a Partially Ordered Set  $P$  and the Covering Theorems.*—By the zero element of  $P$  is meant an element which is in the relation  $<$  to every other element of  $P$ . If  $P$  contains no zero we may always adjoin one and call  $P + 0$  the enlarged partially ordered set. From the results of section 2 we get: *If  $P$  is a partially ordered set with a denumerable separating system which contains a zero, and if  $P'$  is a subset of  $P$  which is lower inductive of denumerable power having the additional property that whenever  $x \neq 0$  is an element of  $P'$  there is a  $y < x$  in  $P'$ , then  $0$  is an element of  $P'$ .*

This theorem yields the Borel covering theorem for completely separable spaces, i.e., that in a space  $S$  with a denumerable basis, from any covering by open sets it is possible to extract a denumerable number of open sets which alone cover  $S$ . Take  $P$  to be the set of all closed subsets of  $S$ , and  $P'$  to be the set of closed subsets of  $S$  which are the complementary sets to the sum of an at most denumerable number of open sets belonging to the covering. The conditions of the theorem hold, and the conclusion means that the vacuous set is the complement of a denumerable number of the open sets in the covering.<sup>3</sup>

*Let  $P$  have a denumerable strong separating system, be lower inductive of denumerable power and have no zero element. Let  $P'$  be a subset of  $P + 0$*

such that 1) if  $x \in P$  there exists an  $x' \in P'$  such that  $x \not\leq x'$ , and 2) if  $x, y$  are elements of  $P'$ , there exists a  $z \in P'$  such that  $z \leq x$  and  $z \leq y$ . Under these conditions zero is an element of  $P'$ . This theorem yields the Heine-Borel covering theorem for compact completely separable spaces. In the application the lower inductiveness of  $P$  is a consequence of the Cantor product theorem.

4. *Topological Spaces.*—Let  $P$  be a partially ordered set with a unit element having a strong separating system  $\mathfrak{M}$  of upper sections. We assume 1) for any two distinct members of  $\mathfrak{M}$ , neither is a subset of the other, and 2) for each pair of elements,  $x, y$  of  $P$ , and each  $U$  of  $\mathfrak{M}$  whenever neither  $x$  nor  $y$  is an element of  $U$ , there is a  $z$  in  $P$  such that  $x \leq z, y \leq z$ , and  $z$  is not an element of  $U$ .

We now define a topological space  $T$  as follows: The points of  $T$  are the members of  $\mathfrak{M}$ . A basis of closed sets of  $T$  is formed by the point sets  $T_x$ , where  $x$  is an element of  $P$ , and  $T_x$  the set of upper sections in  $\mathfrak{M}$  containing  $x$ . If  $M$  is a subset of  $T$  we define the closure  $\overline{M}$  to be the product of all sets  $T_x$  which contain  $M$ . It may readily be verified that a) if  $p$  is a point, then  $\overline{p} = p$ , b)  $\overline{M + N} = \overline{M} + \overline{N}$ , c)  $\overline{\overline{M}} = \overline{M}$ , d) the closure of the vacuous set is the vacuous set. The basis of closed sets  $T_x$  is in one-to-one correspondence with  $P$  preserving the order relations of  $<$  in  $P$  and inclusion in  $T$ .

<sup>1</sup> A subset  $L$  of a partially ordered set  $P$  is called a lower section if whenever  $x$  is an element of  $L$ ,  $y < x$  implies that  $y$  is also an element of  $L$ . In an analogous way upper sections are defined.

<sup>2</sup> If the topological space  $S$  has a basis of open sets  $\{0\}$  of power  $M$ , and if  $P$  is any partially ordered set of some (not necessarily all) closed subsets of  $S$ , then  $P$  has a strong separating system of power at most  $M$ . We obtain one if with each open set  $0$  of  $\{0\}$  we associate the set  $U_0$  of all closed sets which are elements of  $P$  and have a non-vacuous intersection with  $0$ . It should be noted that  $U_0$  is an upper section but is not an ideal even in the case that  $P$  consists of all closed subsets of  $S$ .

<sup>3</sup> The arguments about partially ordered sets with denumerable separating systems do not require the theory of transfinite numbers. The covering theorems for higher powers follow from general theorems about partially ordered sets with separating systems of higher powers.

## ON THE DISTRIBUTION OF NORMAL POINT GROUPS

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Let  $-1 \leq x_1 < x_2 < \dots < x_n \leq 1$  be  $n$  real numbers, and let us write  $\omega_n(x) = \prod_{k=1}^n (x - x_k)$ . Consider the unique polynomial  $f_n(x)$  of degree not exceeding  $2n - 1$  such that

$$f(x_k) = y_k, f'(x_k) = 0.$$

$f_n(x)$  is called the step parabola. It is well known<sup>1</sup> that

$$f_n(x) = \sum_{k=1}^n y_k l_k^2(x) v_k(x) = \sum_{k=1}^n y_k h_k(x) \quad (1)$$

where

$$l_k(x) = \frac{\omega_n(x)}{\omega_n'(x_k)(x - x_k)} \text{ and } v_k(x) = 1 - 2l_k'(x_k)(x - x_k).$$

Thus the linear function  $v_k(x)$  is 1 at  $x_k$  and 0 at

$$X_k = x_k + \frac{\omega_n'(x_k)}{\omega_n''(x_k)} = x_k + \frac{1/2}{\sum_{k \neq v} \frac{1}{x_k - x_v}}.$$

The system  $X_1, X_2, \dots, X_n$  is called by Fejér<sup>2</sup> the conjugate point system of  $x_1, x_2, \dots, x_n$ . If all the  $X_i$  are outside  $-1, +1$ , Fejér calls the point group normal. He pointed out that the roots of many of the classical polynomials are normal, e.g., the roots of the Tchebicheff and Legendre polynomials.

Fejér<sup>3</sup> proved that if  $x_1, x_2, \dots, x_n$  is a normal point group then  $\lim_{n \rightarrow \infty} (x_{i+1} - x_i) = 0$ . Turán and I<sup>4</sup> improved this to

$$x_{i+1} - x_i < \frac{c_1}{n\sqrt{1 - x_i^2}}.$$

Recently I proved that for the  $x_i$  satisfying  $-1 + c_2 < x < 1 - c_2$

$$x_{i+1} - x_i = \frac{\pi}{n\sqrt{1 - x_i^2}} + O\left(\frac{1}{n^{3/2}}\right). \quad (2)$$

It seems likely that if  $-1 + c_2 < x < 1 - c_2$  then

$$x_{i+1} - x_i = \frac{\pi}{n\sqrt{1 - x_i^2}} + O\left(\frac{1}{n^2}\right).$$

Let now  $-1 = z_1 < z_2 < \dots < z_n < 1$  be the roots of the polynomial  $P_n(z) + P_{n-1}(z)$  (where  $P_n(z)$  denotes the  $n$ th Legendre polynomial). It is known<sup>5</sup> that  $Z_2 = Z_3 = \dots = Z_n = 1$ , and  $Z_1 < -1$  (the  $Z_i$  are the conjugate points); i.e., this polynomial is barely normal. It is also easy to show that if for a certain point group  $z_1 = -1$  and  $Z_2 = \dots = Z_n = 1$ , then

$$P_n(z) + P_{n-1}(z) = \prod_{i=1}^n (z - z_i).$$

Thus  $P_n(z) + P_{n-1}(z)$  is characterized by this property. Now we prove the following THEOREM 1: *Let  $-1 \leq x_1 < x_2 < \dots < x_n \leq 1$  be a normal point group. Then*

$$z_i \leq x_i \leq -z_{n-i}.$$

It is easy to see that these limits are the best possible since the point group  $-z_i$  is also normal.

*Proof.* It will be sufficient to prove that

$$z_i \leq x_i.$$

We prove the following stronger result: Suppose  $-1 \leq x_1 < x_2 < \dots < x_n \leq 1$  is such that  $X_i$  does not fall in the interval  $(x_i, 1)$  (we will refer to this property as  $A$ ). Then

$$z_i \leq x_i.$$

Let us investigate the point group satisfying  $A$  and for which  $x_i$  is as small as possible. It is easy to see that such a point group exists.<sup>6</sup> Now we prove that this point group is  $z_1, z_2, \dots, z_n$ , and this will complete our proof. Suppose that this is not true. Then either  $x_1 \neq -1$  or there exists an  $x$ , say  $x_j$ , such that  $X_j \neq 1$ . Suppose first  $x_1 \neq -1$ . Consider the point group  $-1 < x_1 - \epsilon_1 < x_2 < \dots < x_n \leq 1$  ( $\epsilon_1$  sufficiently small). A simple calculation shows that the new point group also satisfies  $A$ , and in fact the conjugate points which were not less than 1 increased in absolute value. Thus if we denote by  $X'_1, X'_2, \dots, X'_n$  the conjugate points of  $x_1 - \epsilon_1, x_2, \dots, x_n$ , we have  $X'_i \neq 1$ . Consider now the point group  $x_1 - \epsilon_1, x_2, \dots, x_i - \epsilon_i, \dots, x_n$  ( $\epsilon_i$  sufficiently small). A simple calculation shows that this point group also satisfies  $A$ , which contradicts the minimum property of  $x_i$ . In the second case we consider the point group  $x_1, x_2, \dots$

$x_j = e_j, \dots, x_n$  ( $e_j$  sufficiently small) which also satisfies  $A$  and the whole proof goes through as before.

Let  $\varphi(x)$  be any continuous function in  $(-1, +1)$ , and let

$$\begin{array}{ccccccc} & & x_1^{(2)} & x_1^{(1)} & x_2^{(2)} & & \\ & \cdot & \cdot & \cdot & \cdot & \cdot & \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \end{array}$$

be any infinite sequence of normal point systems. (Fejér<sup>7</sup> calls a sequence of point groups strongly normal if for any  $k$  and  $n$ ,  $v_k(x) \geq c_k$ ,  $-1 \leq x \leq 1$  ( $c_k$  independent of  $k$  and  $n$ )). Consider the polynomial  $f_n(x)$  of degree not greater than  $2n - 1$  for which

$$f_n(x_i^{(n)}) = \varphi(x_i^{(n)}) \text{ and } f_n'(x_i^{(n)}) = 0.$$

By (1),

$$f_n(x) = \sum_{k=1}^n y_k h_k(x).$$

Fejér<sup>8</sup> conjectured that

$$\lim_{n \rightarrow \infty} f_n(x) = \varphi(x)$$

uniformly in  $(-1, +1)$ . Recently I succeeded in proving that

$$\lim_{n \rightarrow \infty} f_n(x) = \varphi(x), \quad -1 + \epsilon < x < 1 - \epsilon$$

uniformly for every  $\epsilon > 0$ . In fact it suffices to suppose that the sequence of point groups is normal.

The proof of this result is not quite simple (it uses (2)) so that we do not give it here.

In a previous paper<sup>9</sup> Turán and I proved that if  $x_1, x_2, \dots, x_n$  is a normal point group, then

$$\max_{-1 + \epsilon < x < 1 - \epsilon} \prod_{i=1}^n (x - x_i) < \frac{c_1 \sqrt{n}}{2^n}.$$

By using Theorem 1, I can prove that

$$\max_{-1 \leq x \leq 1} \prod_{i=1}^n (x - x_i) < \frac{c_1 \sqrt{n}}{2^n}.$$

<sup>7</sup> L. Fejér, "Lagrangesche Interpolation und die zugehörigen konjugierten Punkte," *Math. Ann.*, 106, 1-55 (1932). See also "On the Characterization of Some . . .," *Amer. Math. Monthly*, 41, 1-14 (1934).

<sup>8</sup> L. Fejér, *Ibid.*, p. 8.



<sup>1</sup> L. Fejér, *Ibid.*, p. 27.

<sup>2</sup> P. Erdős and P. Turán, "On Interpolation, II," *Ann. Math.*, 39, 702-724 (1938).

<sup>3</sup> L. Fejér, *Ibid.* p. 32.

<sup>4</sup> Denote by  $a_i$  the lower limit of the  $x_i$ . To prove the existence of the point group in question it suffices to show that there exists a  $\delta$  such that if  $x_1, x_2, \dots, x_n$  is a point group satisfying  $A$  and for which  $x_i - a_i < \epsilon_i$  ( $\epsilon_i$  sufficiently small), then  $x_{r+1} - x_r > \delta, r = 1, 2, \dots, n-1$ . This is not difficult.

<sup>5</sup> L. Fejér, *Amer. Math. Monthly*, 41, 8 (1934).

<sup>6</sup> L. Fejér, "On the Characterization of Some . . .," *Amer. Math. Monthly*, 41, 13 (1934).

<sup>7</sup> P. Erdős and P. Turán, *Ibid.*

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## THE EFFECT OF AN "EMOTIONAL STATE" ON THE INITIAL STAGES OF ACQUISITION IN A CONDITIONED OPERANT RESPONSE

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In a recent study we have been concerned with an analysis of the acquisition, extinction and spontaneous recovery of a conditioned operant response in the white rat.<sup>1</sup> The apparatus used in this experiment consists of a simple runway, at one end of which is a starting box and at the other end a food box. The measure of response chosen for analysis is termed the latent period. This period is defined as the time taken by the rat to leave the starting box, after the door is opened, before he traverses the runway to food. A complete account of the experimental procedure is contained in the previous report. By this procedure it was found that the curve of acquisition begins with a high value of log latent period on the first trial ( $\log 71 = 1.85$ ) and falls off with an increase in the number of trials; by the fifteenth trial it seems to be approaching a final limiting value ( $\log 2.8 = 0.45$ ). The curve *B* of figure 1 represents this result; it is the curve drawn through the acquisition data.

An important part of the original paper is concerned with a rational formulation of acquisition, extinction and spontaneous recovery. It soon became apparent in the course of this aspect of the work that a term would be required which would state conditions for the initial step of acquisition. This requirement is due to the fact that the beginning of conditioning may be influenced by a number of factors. In the first place, it is possible that an animal may come to the experimental situation with an already appreciable amount of conditioning in a given performance. Secondly, it may be true, in the case of another animal or another situation, that the first few

trials do not contribute directly to the conditioning, but only to the animal's "emotional adjustment" to the experimental apparatus. In either case attention must be paid to behavior at the beginning of acquisition. In view of this consideration, the equation (10) for acquisition in the earlier paper contains a term,  $P_m$ , which corresponds to the latent period identified as occurring at the beginning of conditioning.

The present report presents experimental evidence for the validity of these ideas. It is shown that, under conditions which lead initially to "emotional maladjustment" (1) no conditioning takes place during the

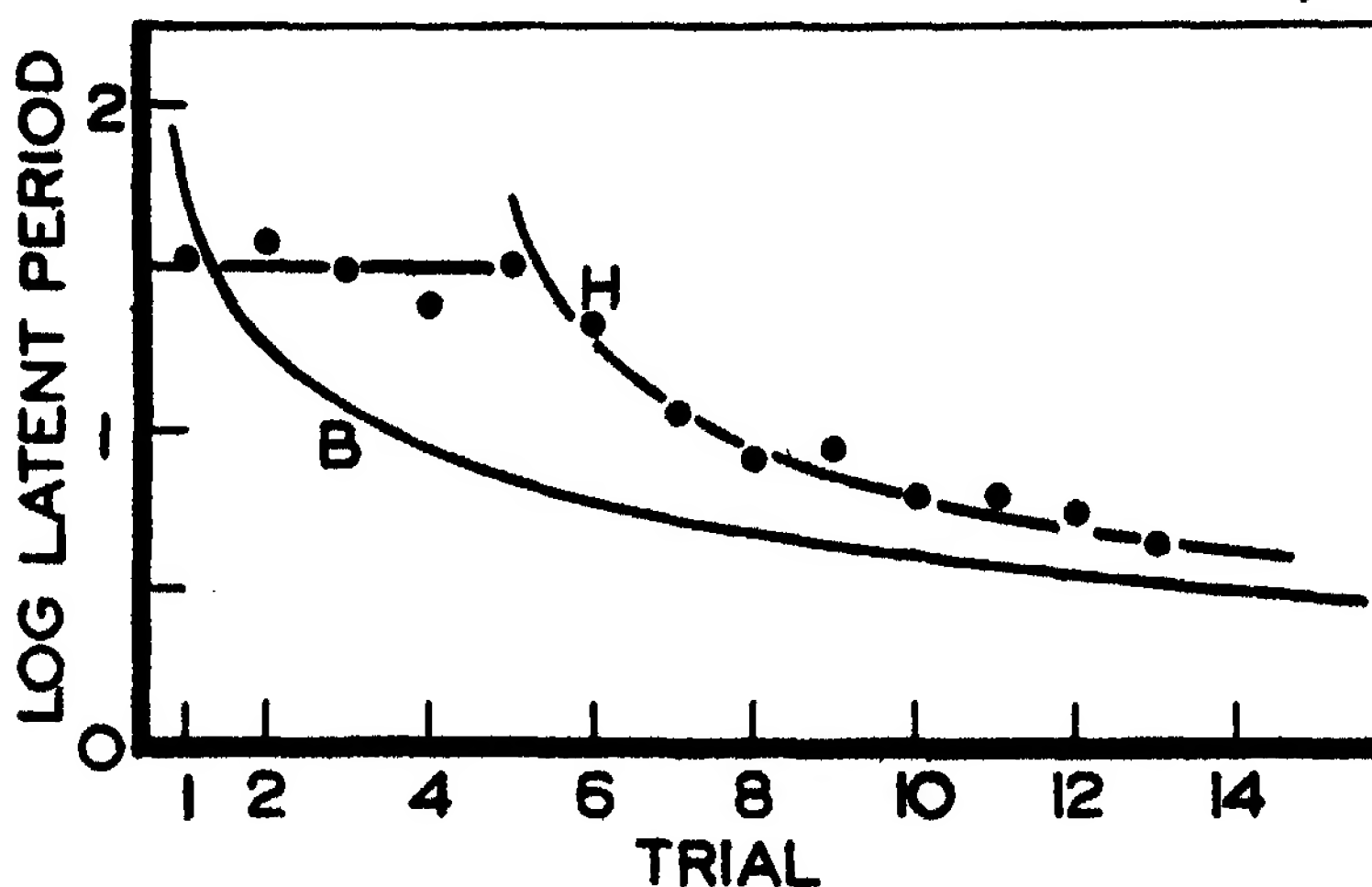


FIGURE 1

Acquisition data for the "handled" animals (*H*) of this experiment and the "box" animals (*B*) of the earlier experiment. The solid circles are the experimental points for group *H*. Curve *H* beyond trial five is similar in shape to curve *B*.

first few trials. However (2) when conditioning does get under way, it takes place at a rate comparable to the one observed under conditions of little "maladjustment."

The results of the present experiment were obtained in the course of the research reported earlier. In the original experiment the food and the entrance boxes were interchangeable, and the animal was shifted at the end of each trial by moving the food box to the entrance position. In a short series on nine animals this procedure was not followed. For these experiments the food and entrance boxes were set permanently in the apparatus. Each box contained a door in front; the sides and back were constructed

of wire mesh, and the top was open to allow for the placing or lifting of the animal. Under these circumstances the movement of the rat from the food box to the entrance box at the end of a trial was effected by hand. In other details the experiments were similar to those reported in the earlier paper.<sup>2</sup>

The data for the "handled" animals are shown in curve *H* of figure 1. In this figure it may be observed that the "handled" animals show little improvement until trial 5. From that trial on, however, they follow a course of acquisition which is identical with the one given by the animals moved in a box (curve *B*). The similarity between group *H*'s data ("handled" animals) beyond the first five trials to group *B*'s data ("box" animals) is shown by the fact that curve *H* is curve *B* moved a distance of 4 trials along the abscissa. Presumably "handling" in the case of group *H* introduced an emotionally disturbing condition which effectively protracted the time required for habituation to the experiment. If this be so, it is noteworthy that once this effect has disappeared the course of conditioning is unaffected. Thus it would seem that under our experimental conditions an initial "emotional state" due to "handling" has the sole effect of delaying the time of "getting started." Whether or not the rate of acquisition would be changed if the emotional disturbance had come at some other time than in the initial stages is a problem on which we have no evidence at present.

The results of our experiment have an important theoretical implication. They seem to demonstrate the necessity for the term  $P_m$  in our acquisition equation of the earlier paper. The presence of this term insures the identification of the response at the beginning of acquisition and thus eliminates from consideration a number of irrelevant initial activities, which have no significance for the problem. Among these irrelevant activities are some which may be loosely categorized as "emotion" and "adjustment to the experimental situation."

*Summary.*—We have carried out an experiment on the acquisition of a conditioned operant response which is similar in all details except one to a research already reported. The difference between the two experiments consists in the fact that the animals of the present investigation were "handled" between trials, while the original animals were not. "Handling" seems to introduce an "emotional state" which delays the beginning of conditioning. However, once conditioning has begun, the rates of acquisition for both groups are similar.

<sup>1</sup> Graham, C. H., and Gagné, R. M., *Jour. Exper. Psychol.*, 26, 251(1940).

<sup>2</sup> The animals of this group (*H*) were given 13 trials of acquisition rather than the 15 required of the original group (*B*). The average log latent period values in seconds for the nine animals of *H* are, in order from trial one to trial thirteen: 1.51, 1.58, 1.50, 1.39,

1.51, 1.34, 1.05, 0.92, 0.95, 0.81, 0.81, 0.78, 0.64. It may be seen that the values of the first five trials are below the latent period for the first trial of group *B* (1.85). The initial trial is theoretically susceptible to the influence of many variables, and this observation would seem to give further reason for discounting the reliability of the initial stages of acquisition. As the data show, the later stages are highly reproducible.

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## NOTE ON THE PROBLEM OF THE DISTRIBUTION OF STRESS IN A THIN STIFFENED ELASTIC SHEET

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1. *Introduction.*—One of the practically interesting problems in the Theory of Plane Stress for which no solution has hitherto been given is the following: A thin flat stiffened sheet is acted upon by concentrated edge forces in the plane of the sheet. If the sheet were unstiffened the stresses at the points of load application would be infinite. To avoid these infinite stresses, stiffening rods are attached to the sheet such that the loads are applied to the ends of the stiffeners. Due to their finite cross-sectional area the stresses in them are finite and then for reason of continuity the normal stresses in the sheet are also finite. The stiffeners unload themselves gradually into the sheet by means of the shear acting between sheet and stiffener. At a certain distance from the edges of the sheet the stresses become sufficiently reduced to make stiffening further inwards unnecessary. The question arises as to what dimensions the stiffeners should have so that the shear load per unit of length applied to the sheet and the stresses in the unstiffened part of the sheet remain below prescribed limits.

In the present note a basic case of this problem is considered. An integral equation is derived for the distribution of stress along the stiffener. This equation is of the same type as the integral equations occurring in the theory of aerofoils of finite span and can be treated by the same methods.

Without calculation a noteworthy result concerning the dependence of solution on the relative dimensions of sheet and stiffener is obtained in this paper.

The actual solutions of the integral equations remain to be calculated.

2. *Formulation of the Problem.*—Consider a sheet of thickness  $t$  of infinite extension on one side of its straight edge. A concentrated force  $F$  is acting on and normal to the boundary. A stiffener of length  $l$  and cross-sectional area  $A$  (which may be variable) is attached to the sheet so that the axis of the stiffener coincides with the direction of the force  $F$  and the load is introduced into the stiffener. A coördinate system  $x, y$  in the plane

of the sheet is chosen so that the  $y$ -axis conforms with the boundary of the sheet, and part of the  $x$ -axis with the axis of the stiffening rod.

The stiffener then is loaded at one end by the force  $F$ , along its length by an unknown shear distribution  $s(x)$  and at the other end by a force  $F^* = F - \int_0^l s(x)dx$ . Because of equilibrium the shear  $s$  is also acting on the sheet as a line distribution of body forces<sup>1</sup> and the force  $F^*$  as a concentrated body force.

The variation of  $s$  must be such that at any point the normal strain  $\epsilon_x(\xi, 0)$  in the sheet due to the presence of the body forces equals the strain  $\epsilon(\xi)$  in the stiffener due to its loading  $F - \int_0^\xi s(x)dx$  to the left of the point  $\xi$ . If the modulus of elasticity of the sheet is denoted by  $E_s$ , that of the stiffener by  $E_{st}$ , and if  $K(x, \xi)$  stands for the strain  $\epsilon_x(\xi, 0)$  due to a unit body force applied at the point  $x$ , one has

$$\epsilon_x(\xi, 0) = \frac{1}{tE_s} \left\{ \int_0^l s(x)K(x, \xi)dx + F^*K(l, \xi) \right\} \quad (1)$$

$$\epsilon(\xi) = \frac{1}{AE_{st}} \left\{ F - \int_0^\xi s(x)dx \right\}. \quad (2)$$

The condition that  $\epsilon_x(\xi, 0) = \epsilon(\xi)$  can then be written

$$F - \int_0^\xi s(x)dx = \frac{AE_{st}}{tE_s} \left\{ F^*K(l, \xi) + \int_0^l s(x)K(x, \xi)dx \right\}. \quad (3)$$

In order to determine  $s(x)$  from this equation it is necessary to know the form of the kernel  $K$ . Since the explicit solution of the problem of the stress distribution in a semi-infinite sheet due to a concentrated body force is known,<sup>2</sup>  $K$  can be written down as follows:

$$\pi K(x, \xi) = \frac{C_1}{\xi - x} + \frac{C_2 x \xi}{(\xi + x)^3} + \frac{C_3 x + C_4 \xi}{(\xi + x)^2} + \frac{C_5}{\xi + x} \quad (4)$$

$$\left. \begin{aligned} 4C_1 &= 2 + 3\nu - \nu^2, & C_2 &= 1 + \nu, \\ 4C_3 &= (1 - \nu)(1 - 3\nu), & 4C_4 &= (1 - \nu)(3 - \nu), & 2C_5 &= 1 + \nu \end{aligned} \right\}. \quad (5)$$

This kernel  $K$  becomes singular when  $x$  approaches  $\xi$ . The singularity is of such nature that the principal value of the integral in (3) has to be taken, in which case the integral converges.

Introducing instead of the shear distribution  $s$  the stiffener stress  $\sigma$ ,

$$\left. \begin{aligned} A\sigma &= F - \int_0^\xi s(x)dx \\ (A\sigma)_l &= F^* = F - \int_0^l s(x)dx \end{aligned} \right\} \quad (6)$$

(3) becomes

$$\sigma(\xi) = \frac{E_{st}}{tE_s} \left\{ A(l)\sigma(l)K(l,\xi) - \int_0^l [A(x)\sigma(x)]' K(x,\xi)dx \right\}. \quad (7)$$

It is convenient to distinguish the two cases of infinite and of finite stiffener length  $l$ .

1°. When  $l$  is infinite the stress  $\sigma(l)$  is obviously zero and one has:

$$\sigma(\xi) + \int_0^\infty \left[ \frac{A(x)E_{st}}{tE_s} \sigma(x) \right]' K(x,\xi)dx = 0. \quad (7a)$$

The arbitrary factor in the solution of this homogeneous equation is determined from the condition

$$\sigma(0)A(0) = F. \quad (8a)$$

2°. When  $l$  is finite there arises a certain difficulty from the fact that on the right side of equation (7) the first term approaches an infinite value when  $\xi$  approaches  $l$  and  $A(l)\sigma(l)$  is different from zero. It seems, however, that one has to assume in any case

$$\sigma(l)A(l) = 0, \quad (9)$$

for if  $\sigma(l)A(l)$  were different from zero, that is  $F^*$  different from zero, there would be a concentrated body force acting in the sheet immediately at the end section of the stiffener. This would cause an infinite strain at this point of the sheet, which in turn would give infinite sheet and stiffener stresses at the end section of the stiffener; hence because of the finite area over which this stress is acting there would be an infinitely large force acting through the cross-section of the stiffener. This, however, is physically impossible. It appears thus that the integral equation to be solved is

$$\sigma(\xi) + \int_0^l \left[ \frac{A(x)E_{st}}{tE_s} \sigma(x) \right]' K(x,\xi)dx = 0. \quad (7b)$$

If the foregoing argument is correct there must be two linearly independent solutions of this homogeneous equation (7b), to satisfy the two end conditions

$$\sigma(0)A(0) = F, \quad \sigma(l)A(l) = 0. \quad (8b)$$



That this is indeed so is made plausible by the following consideration. The singular part of the kernel,  $1/(\xi - x)$  is of the same type as a kernel of the form

$$K(x, \xi) = \begin{cases} \frac{1}{\epsilon^2}, & \xi - \epsilon < x < \xi, \\ -\frac{1}{\epsilon^2}, & \xi < x < \xi + \epsilon, \\ 0, & x < \xi - \epsilon, \quad \xi + \epsilon < x. \end{cases} \quad (10)$$

In this simplified case the integral equation (7b) is transformed—when  $\epsilon$  approaches zero—into the second order differential equation<sup>3</sup>

$$\sigma(\xi) - \left[ \frac{A(\xi)E_{st}}{tE_s} \sigma(\xi) \right]' = 0, \quad (11)$$

which has indeed two linearly independent solutions.

It remains, however, to be *proved* that the equation (7b) has the same property.

3. *The Inverse Problem.*—Instead of prescribing the law of variation of stiffener area  $A$  and asking for the corresponding stress distribution, it is also of some interest from a design point of view to ask how  $A$  has to vary in order to produce a prescribed distribution of shear  $s$  between sheet and stiffener. Considering equation (3) one has, with  $F^*$  equal to zero,

$$A(\xi) = \frac{tE_s}{E_{st}} \frac{F - \int_0^\xi s(x)dx}{\int_0^l s(x)K(x, \xi)dx}. \quad (12)$$

This shows that the inverse problem of expressing the area in terms of a prescribed shear distribution admits a very simple solution by direct integration.

4. *Results of a Dimensional Analysis.*—Without actually determining the solutions of the integral equations (7a) and (7b) statements can be made, helpful for the analysis of experimental data, concerning the dependence of the solution on the parameter  $A$ ,  $t$ ,  $l$ ,  $E_{st}$  and  $E_s$ . Considering for the sake of simplicity only cases of constant cross-sectional area  $A$ , and considering separately the equations for infinite and finite length  $l$ , the following facts are true:

1°. For infinite  $l$  a change of variables

$$z = \frac{E_s}{E_{st}} \frac{t}{A} x, \quad \zeta = \frac{E_s}{E_{st}} \frac{t}{A} \xi, \quad \sigma(x) = \sigma^*(z) \quad (13)$$

transforms equation (7a) into

$$\sigma^*(\zeta) + \int_0^\infty [\sigma^*(z)]' K(z, \zeta) dz = 0 \quad (14)$$

with

$$A\sigma^*(0) = F. \quad (15)$$

This means that the solution has the form

$$\sigma^*(z) = \frac{F}{A} f(z)$$

or, expressed in terms of the original variable  $x$ ,

$$\sigma(x) = \frac{F}{A} f\left(\frac{E_s}{E_{st}} \cdot \frac{tx}{A}\right). \quad (16)$$

The result (16) indicates that if only the stress distribution for one particular set of values of  $A$ ,  $l$ ,  $E_s$  and  $E_{st}$  is determined, it is then also known for any other values of these quantities.

2°. For finite  $l$  another change of variables

$$z = \frac{x}{l}, \quad \zeta = \frac{\xi}{l} \quad (17)$$

transforms equation (7b) into

$$\sigma^*(\zeta) + \left[ \frac{E_{st}}{E_s} \cdot \frac{A}{tl} \right] \int_0^1 [\sigma^*(z)]' K(z, \zeta) dz = 0. \quad (18)$$

The form of this equation shows that  $\sigma$  can be expressed in the form

$$\sigma(x) = \frac{F}{A} g\left(\frac{x}{l}; \frac{E_{st}}{E_s} \cdot \frac{A}{tl}\right). \quad (19)$$

Formula (19) proves that the type of the stress distribution depends only on the value of one dimensionless quantity  $(E_{st}A/E_s tl)$ . In the limit for  $l \rightarrow \infty$  the function  $g$  in (19) must of course become the corresponding function  $f$  in (16).

5. *Summary.*—An integral equation is derived for the distribution of the stresses arising when a concentrated force is applied to a flat sheet by means of a stiffener. It is seen that the equation is a generalization of one occurring in the theory of aerofoils of finite span. It is shown to be simpler to solve the inverse problem of determining the stiffener sizes for a prescribed stress distribution than to determine the stresses for given dimensions of the structure. It is proved that for constant cross-sectional area of the stiffener the stress distribution depends only on two dimension-

less parameters composed of the six independent dimensions and moduli of elasticity occurring in the problem, and that for an infinite stiffener length only one parameter remains. The method of analysis here presented is of course applicable to more general cases. If, for instance, a sheet with several stiffeners is considered, a system of simultaneous integral equations is obtained instead of the single equation (7).

<sup>1</sup> The idea to represent by a system of body forces the interaction between one part of a structure and another has previously been introduced, in the theory of "Effective Width" problems, by H. Reissner, *Z. Ang. Math. Mech.*, 14, 312 (1934).

<sup>2</sup> E. Melan, *Z. Ang. Math. Mech.*, 12, 343 (1932).

<sup>3</sup> Equations of this sort have previously been obtained by making very strong simplifying assumptions concerning the elastic behavior of the sheet.

## ON SOME PERIODIC PROPERTIES OF THE SYSTEM OF ISOTOPES

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It has been known for a long time that the system of isotopes shows some remarkable regularities which have been the subject of various investigations.<sup>1</sup> It will be the task of this paper to discuss some additional regularities which apparently have not yet been considered. Since the most important group of isotopes are those with even charge and mass numbers, these will be first discussed.

As table 1 shows, these isotopes may be arranged in consecutive series each of which is characterized by a definite value of the isotopic number ( $I$ ) which is defined as the difference between the numbers of neutrons and protons, i.e.,  $N-P$  or  $A-2Z$ , where  $A$  is the mass number and  $Z$  the charge number.<sup>2</sup> Because  $A$  and  $Z$  are even, the scheme contains only even values of  $I$ . The highest known value of  $I$  is 54 ( $_{92}\text{U}^{238}$ ); only one unstable nucleus with even  $Z$  and even  $A$  is known<sup>3</sup> for  $2 < Z < 82$ .

In the series of table 1 the charge number increases by two and the mass number by four from one place to the next. As may be seen from table 1, the successive series or periods, as we may call them, show regular lengths if we disregard a few very weak and in some cases perhaps dubious isotopes which with the only exception of  $_{20}\text{Ca}^{48}$  and  $_{32}\text{S}^{144}$  maybe recorded in table 1 as either immediately preceding or immediately succeeding the periods themselves. There are ten such isotopes of which, however, only four are to be found in the last Report of the International Committee on

TABLE 1

## STABLE NUCLEI WITH EVEN CHARGE AND MASS NUMBERS

(The number below the chemical symbol indicates the relative abundance in per cent. The symbols n and p indicate that the nucleus remains stable after addition of a neutron or proton, respectively)

ISOTOPIC NUMBER	PRE- CEDING	I	II	III	IV	V	VI	VII	VIII	IX	X	SUC- CEDING
I = 0		${}^2\text{He}^4$ 100 n (?)	${}^4\text{Be}^8$ ? n	${}^6\text{C}^{12}$ 98.9 n	${}^8\text{O}^{16}$ 99.76 n	${}^{10}\text{Ne}^{20}$ 90.0 n	${}^{12}\text{Mg}^{24}$ 77.4 n	${}^{14}\text{Si}^{28}$ 89.6 n	${}^{16}\text{S}^{32}$ 95.0 n	${}^{18}\text{Ar}^{36}$ 0.307 n	${}^{20}\text{Ca}^{40}$ 96.96 n	
I = 2	${}^8\text{O}^{16}$ 0.20 p	${}^{10}\text{Ne}^{22}$ 9.73 p	${}^{12}\text{Mg}^{26}$ 11.1 p	${}^{14}\text{Si}^{30}$ 4.2 p	${}^{16}\text{S}^{34}$ 4.2 p	${}^{18}\text{Ar}^{38}$ 0.06 p	${}^{20}\text{Ca}^{42}$ 0.64 n	${}^{22}\text{Ti}^{48}$ 7.95 n	${}^{24}\text{Cr}^{50}$ 4.49 n	${}^{26}\text{Fe}^{54}$ 6.04 n	${}^{28}\text{Ni}^{58}$ 68.0 n	
I = 4		${}^{16}\text{S}^{36}$ 0.016 p	${}^{18}\text{Ar}^{40}$ 99.632 p	${}^{20}\text{Ca}^{44}$ 2.06 p	${}^{22}\text{Ti}^{48}$ 73.45 n	${}^{24}\text{Cr}^{52}$ 83.77 n	${}^{26}\text{Fe}^{56}$ 91.57 p, n.	${}^{28}\text{Ni}^{60}$ 27.2 n	${}^{30}\text{Zn}^{64}$ 50.9 n			
I = 6	${}^{20}\text{Ca}^{46}$ 0.0033	${}^{22}\text{Ti}^{50}$ 5.34 p	${}^{24}\text{Cr}^{54}$ 2.30 p	${}^{26}\text{Fe}^{58}$ 0.28 p	${}^{28}\text{Ni}^{62}$ 3.8 p	${}^{30}\text{Zn}^{66}$ 27.3 n	${}^{32}\text{Ge}^{70}$ 21.2 n	${}^{34}\text{Se}^{74}$ 0.9 n	${}^{36}\text{Kr}^{78}$ 0.35 n			
I = 8		${}^{28}\text{Ni}^{64}$ 0.9 p	${}^{30}\text{Zn}^{68}$ 17.4 p	${}^{32}\text{Ge}^{72}$ 27.3 n	${}^{34}\text{Se}^{76}$ 9.5 n	${}^{36}\text{Kr}^{80}$ 2.01 n	${}^{38}\text{Sr}^{84}$ 0.56 n	${}^{40}\text{Zr}^{88}$ ... n	${}^{42}\text{Mo}^{92}$ 15.5 n			${}^{44}\text{Ru}^{96}$ 5
I = 10		${}^{30}\text{Zn}^{70}$ 0.5 p	${}^{32}\text{Ge}^{74}$ 37.1 p	${}^{34}\text{Se}^{78}$ 24.0 p	${}^{36}\text{Kr}^{82}$ 11.53 n	${}^{38}\text{Sr}^{86}$ 9.86 n	${}^{40}\text{Zr}^{90}$ 48 n	${}^{42}\text{Mo}^{94}$ 8.7 n	${}^{44}\text{Ru}^{98}$ ? n	${}^{46}\text{Pd}^{102}$ 0.8 n	${}^{48}\text{Cd}^{106}$ 1.4 n	
I = 12		${}^{32}\text{Ge}^{76}$ 6.5 p	${}^{34}\text{Se}^{80}$ 48.0 p	${}^{36}\text{Kr}^{84}$ 57.10 p	${}^{38}\text{Sr}^{88}$ 82.56 p	${}^{40}\text{Zr}^{92}$ 22 p	${}^{42}\text{Mo}^{96}$ 16.8 n	${}^{44}\text{Ru}^{100}$ 14 p, n.	${}^{46}\text{Pd}^{104}$ 9.3 n	${}^{48}\text{Cd}^{108}$ 1.0 n	${}^{50}\text{Sn}^{112}$ 1.1 n	
I = 14		${}^{34}\text{Se}^{82}$ 9.3 p	${}^{36}\text{Kr}^{86}$ 17.47 p	${}^{38}\text{Sr}^{90}$ ... p	${}^{40}\text{Zr}^{94}$ 17 p	${}^{42}\text{Mo}^{98}$ 25.4 p (?)	${}^{44}\text{Ru}^{102}$ 30 p	${}^{46}\text{Pd}^{106}$ 27.2 p	${}^{48}\text{Cd}^{110}$ 12.8 n			${}^{50}\text{Sn}^{114}$ 0.8 n
I = 16		${}^{40}\text{Zr}^{98}$ 1.5 p	${}^{42}\text{Mo}^{100}$ 8.6 p	${}^{44}\text{Ru}^{104}$ 17 p	${}^{46}\text{Pd}^{108}$ 26.8 p	${}^{48}\text{Cd}^{112}$ 24.2 p, n	${}^{50}\text{Sn}^{116}$ 15.5 n	${}^{52}\text{Te}^{120}$ <0.1 n	${}^{54}\text{X}^{124}$ 0.094 n			
I = 18		${}^{46}\text{Pd}^{110}$ 13.5 p	${}^{48}\text{Cd}^{114}$ 28.0 p	${}^{50}\text{Sn}^{118}$ 22.5 n	${}^{52}\text{Te}^{122}$ 2.9 n	${}^{54}\text{X}^{126}$ 0.088 n	${}^{56}\text{Ba}^{130}$ 0.101 n					

I = 20	$^{116}\text{Cd}$ 7.3	$^{120}\text{Sn}$ 28.5	$^{132}\text{Te}$ 4.5	$^{134}\text{X}$ 1.90	$^{138}\text{Ba}$ 0.097	$^{140}\text{Ce}$ <1	$^{152}\text{Sm}$ 3
I = 22	$^{120}\text{Sn}$ 5.5	$^{124}\text{Te}$ 19.0	$^{134}\text{X}$ 4.07	$^{138}\text{Ba}$ 2.42	$^{142}\text{Ce}$ <1	$^{154}\text{Nd}$ 25.95	
I = 24	$^{124}\text{Sn}$ 6.8	$^{128}\text{Te}$ 32.8	$^{138}\text{X}$ 26.96	$^{142}\text{Ba}$ 7.81	$^{146}\text{Ce}$ 90	$^{158}\text{Nd}$ 22.6	$^{162}\text{Gd}$ 0.2
I = 26	$^{128}\text{Te}$ 33.1	$^{132}\text{X}$ 10.54	$^{142}\text{Ba}$ 71.66	$^{146}\text{Ce}$ 10	$^{150}\text{Nd}$ 16.5	$^{162}\text{Sm}$ 14	$^{166}\text{Dy}$ 0.1
I = 28	$^{132}\text{X}$ 8.95	$^{136}\text{Ba}$ ...	$^{146}\text{Ce}$ ...	$^{150}\text{Nd}$ 6.8	$^{154}\text{Sm}$ 26	$^{166}\text{Dy}$ 1.5	$^{170}\text{Yb}$ 0.06
I = 30	$^{136}\text{Nd}$ 5.95	$^{140}\text{Sm}$ 20	$^{154}\text{Gd}$ 22.6	$^{158}\text{Dy}$ 24.6	$^{162}\text{Er}$ 35.2	$^{174}\text{Yb}$ 2	$^{178}\text{Hf}$ 0.3
I = 32	$^{140}\text{Gd}$ 15.7	$^{144}\text{Dy}$ 27.6	$^{158}\text{Er}$ 29.3	$^{162}\text{Yb}$ 23.5	$^{166}\text{Hf}$ 5	$^{178}\text{W}$ ~0.2	$^{182}\text{Os}$ 0.018
I = 34	$^{144}\text{Dy}$ ...	$^{148}\text{Er}$ 9.8	$^{162}\text{Yb}$ 37.2	$^{166}\text{Hf}$ 28	$^{170}\text{W}$ 22.6	$^{182}\text{Os}$ 1.59	
I = 36	$^{166}\text{Yb}$ 11.8	$^{170}\text{Hf}$ 30	$^{184}\text{W}$ 30.1	$^{188}\text{Os}$ 13.3	$^{192}\text{Pt}$ 0.8	$^{200}\text{Hg}$ 0.15	
I = 38	$^{170}\text{W}$ 29.8	$^{174}\text{Os}$ 26.4	$^{194}\text{Pt}$ 30.2	$^{198}\text{Hg}$ 10.1			
I = 40	$^{182}\text{Os}$ 41.0	$^{186}\text{Pt}$ 26.6	$^{200}\text{Hg}$ 23.3	$^{204}\text{Pb}$ 1.48			
I = 42	$^{192}\text{Pt}$ 7.2	$^{196}\text{Hg}$ 29.6	$^{210}\text{Pb}$ 23.59	$^{214}\text{Po}$ 100			

Atoms (1938), namely,  ${}_8\text{O}^{18}$ ,  ${}_{44}\text{Ru}^{96}$ ,  ${}_{50}\text{Sn}^{144}$  and  ${}_{76}\text{Os}^{184}$ . The other six were only recently discovered.<sup>4</sup>

If these few exceptions be disregarded, the lengths of the consecutive periods for  $I = 0$  to  $I = 42$  are found to be

10, 10, 8, 8, 8, 10, 10, 8, 8, 6, 6, 6, 8, 8, 8, 6, 6, 6, 6, 4, 4, 4.

From period to period the charge number of the first column jumps by

8, 6, 6, 6, 2, 2, 2, 6, 6, 2, 2, 0, 2, 2, 6, 4, 2, 4, 4, 2, 2.

Table 1 contains only a few gaps, namely

${}_{38}\text{Sr}^{90}$ ,  ${}_{40}\text{Zr}^{88}$ ,  ${}_{56}\text{Ba}^{140}$ ,  ${}_{58}\text{Ce}^{144}$ ,  ${}_{66}\text{Dy}^{166}$ .

The relative abundance is, in general, considerably smaller in the first or in the last occupied column than in the other columns. If we consider the periods from  $I = 6$  to  $I = 36$ , thus omitting the very lowest and very highest ones (on account of the irregularities at the beginning and the instability at the end), we find as an average value for the first column 8.4, for the second 21.2, for the third 23.3, for the next to the last occupied place 6.3 and for the last occupied place 4.1.

We notice also a certain periodicity if we consider which among the isotopes contained in table 1 remain stable after the addition of a proton, and which after the addition of a neutron (cf. the symbols p and n in table 1). Nuclei of the first kind (p) are found in general on the left-hand side, those of the second kind (n) occur mainly in the middle and only occasionally toward the end of the period.<sup>5</sup>

If we compare the course of the values of the relative abundance in two consecutive periods, we often find an indication of some parallelism through parts of the periods. An example is afforded by a comparison of the first five places in the periods  $I = 20$  and  $I = 22$ .

Indications of periodicities similar to those discussed above are also to be found in two other tables which may be constructed by adding either a neutron or a proton to the isotopes of table 1. We find, e.g., as far as stability, relative abundance and half-value period are concerned:

	II	III	IV	V	VI	VII
$I = 23$	${}_{52}\text{Te}^{127}$	${}_{54}\text{X}^{131}$	${}_{56}\text{Ba}^{135}$	${}_{58}\text{Ce}^{139}$	${}_{60}\text{Ne}^{143}$	${}_{62}\text{Sm}^{147}$
	10 h	21.17%	6.59%	2.1 m	13.0%	17%
$I = 25$	${}_{53}\text{Te}^{129}$	.....	${}_{56}\text{Ba}^{137}$	${}_{58}\text{Ce}^{141}$	${}_{60}\text{Ne}^{145}$	${}_{62}\text{Sm}^{149}$
	30 d		11.32%	15 d	9.2%	15%

We observe also a sequence of approximately equal values of the relative abundance in the series  $I = 11$ , namely:





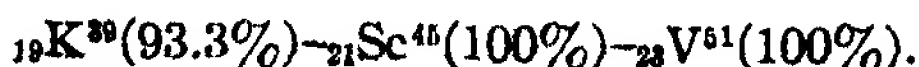
TABLE 2 (Continued)

$r = 15; s = 3 \text{ to } 15 :$	$^{88}\text{Kr}^{78} -$ (0.35)	$^{88}\text{Sr}^{84} -$ (0.56)	$^{90}\text{Zr}^{90} -$ (48)	$^{92}\text{Mo}^{94} -$ (16.8)	$^{94}\text{Ru}^{102} -$ (30)	$^{96}\text{Pd}^{106} -$ (26.8)	$^{98}\text{Cd}^{114}$ (28.0)
	$- ^{100}\text{Sn}^{100} -$ (28.5)	$^{102}\text{Te}^{106} -$ (19.0)	$^{104}\text{X}^{112} -$ (26.96)	$^{106}\text{Ba}^{120} -$ (71.66)	$^{108}\text{Ce}^{144} -$ (?)	$^{110}\text{Nd}^{160}$ (5.95)	
$r = 16; s = 5 \text{ to } 16 :$	$^{92}\text{Mo}^{94} -$ (8.7)	$^{94}\text{Ru}^{100} -$ (14)	$^{96}\text{Pd}^{104} -$ (27.2)	$^{98}\text{Cd}^{112} -$ (24.2)	$^{100}\text{Sn}^{118} -$ (22.5)	$^{102}\text{Te}^{124} -$ (4.5)	$^{104}\text{X}^{130}$ (4.07)
	$- ^{104}\text{Ba}^{124} -$ (7.81)	$^{106}\text{Ce}^{142} -$ (10)	$^{108}\text{Nd}^{148} -$ (6.8)	$^{110}\text{Sm}^{164} -$ (20)	$^{112}\text{Gd}^{180}$ (15.7)		
$r = 17; s = 4 \text{ to } 18 :$	$^{94}\text{Mo}^{92} -$ (15.5)	$^{96}\text{Ru}^{98} -$ (?)	$^{98}\text{Pd}^{104} -$ (9.3)	$^{100}\text{Cd}^{110} -$ (12.8)	$^{102}\text{Sn}^{116} -$ (15.5)	$^{104}\text{Te}^{122} -$ (2.9)	$^{106}\text{X}^{128}$ (1.90)
	$- ^{104}\text{Ba}^{124} -$ (2.42)	$^{106}\text{Ce}^{140} -$ (90)	$^{108}\text{Nd}^{146} -$ (16.5)	$^{110}\text{Sm}^{162} -$ (26)	$^{112}\text{Gd}^{158} -$ (22.6)	$^{114}\text{Dy}^{184} -$ (27.6)	$^{116}\text{Er}^{176}$ (9.8)
	$- ^{118}\text{Yb}^{178}$ (11.8)						
$r = 18; s = 4 \text{ to } 22 :$	$^{96}\text{Ru}^{96} -$ (5)	$^{98}\text{Pd}^{102} -$ (0.8)	$^{100}\text{Cd}^{108} -$ (1.0)	$^{102}\text{Sn}^{114} -$ (0.8)	$^{104}\text{Te}^{120} -$ ( $< 0.1$ )	$^{106}\text{X}^{126} -$ (0.088)	$^{108}\text{Ba}^{132}$ (0.097)
	$- ^{110}\text{Ce}^{120} -$ ( $< 1$ )	$^{112}\text{Nd}^{144} -$ (22.6)	$^{114}\text{Sm}^{160} -$ (5)	$^{116}\text{Gd}^{180} -$ (22.6)	$^{118}\text{Dy}^{182} -$ (24.6)	$^{120}\text{Er}^{188} -$ (29.8)	$^{122}\text{Yb}^{174}$ (37.2)
	$- ^{122}\text{Hf}^{180} -$ (30)	$^{124}\text{W}^{184} -$ (29.8)	$^{126}\text{Os}^{192} -$ (41.0)	$^{128}\text{Pt}^{198} -$ (7.2)	$^{130}\text{Hg}^{204}$ (6.7)		
$r = 19; s = 5, 6,$ 8 to 27	: $\left( \begin{array}{l} ^{100}\text{Cd}^{108} - \\ (1.4) \end{array} \right. - \left( \begin{array}{l} ^{102}\text{Sn}^{112} \\ (1.1) \end{array} \right) - ^{104}\text{X}^{124} - ^{106}\text{Ba}^{130} - ^{108}\text{Ce}^{138} - ^{110}\text{Nd}^{142} - ^{112}\text{Sm}^{148}$						
	$- ^{114}\text{Gd}^{164} -$ (1.5)	$^{116}\text{Dy}^{160} -$ (1.5)	$^{118}\text{Er}^{168} -$ (35.2)	$^{120}\text{Yb}^{172} -$ (23.5)	$^{122}\text{Hf}^{178} -$ (28)	$^{124}\text{W}^{184} -$ (30.1)	$^{126}\text{Os}^{190}$ (26.4)
	$- ^{128}\text{Pt}^{198} -$ (26.6)	$^{130}\text{Hg}^{202} -$ (20.6)	$^{132}\text{Pb}^{208} -$ (52.29)	$^{134}\text{Po}^{214} -$ (weak)	$^{136}\text{Rn}^{220} -$ (weak)	$^{138}\text{Ra}^{226} -$ (100)	$^{140}\text{Th}^{232}$ (100)
	$- ^{142}\text{U}^{238}$ (99.28)						
$r = 20; s = 12 \text{ to } 21 :$	$^{112}\text{Gd}^{152} -$ (0.2)	$^{114}\text{Dy}^{158} -$ (0.1)	$^{116}\text{Er}^{164} -$ (2.0)	$^{118}\text{Yb}^{170} -$ (2)	$^{120}\text{Hf}^{176} -$ (5)	$^{122}\text{W}^{182} -$ (22.6)	$^{124}\text{Os}^{188}$ (13.3)
	$- ^{128}\text{Pt}^{194} -$ (30.2)	$^{130}\text{Hg}^{200} -$ (23.3)	$^{132}\text{Pb}^{206}$ (23.59)				
$r = 21; s = 13 \text{ to } 20 :$	$^{114}\text{Er}^{152} -$ (0.25)	$^{116}\text{Yb}^{168} -$ (0.06)	$^{118}\text{Hf}^{174} -$ (0.3)	$^{120}\text{W}^{180} -$ ( $\sim 0.2$ )	$^{122}\text{Os}^{186} -$ (1.59)	$^{124}\text{Pt}^{192} -$ (0.8)	$^{126}\text{Hg}^{198}$ (10.1)
	$- ^{132}\text{Pb}^{204}$ (1.48)						
$r = 22; s = 16, 18 :$	$^{126}\text{Os}^{184} -$ (0.018)	$^{128}\text{Hg}^{196}$ (0.15)					

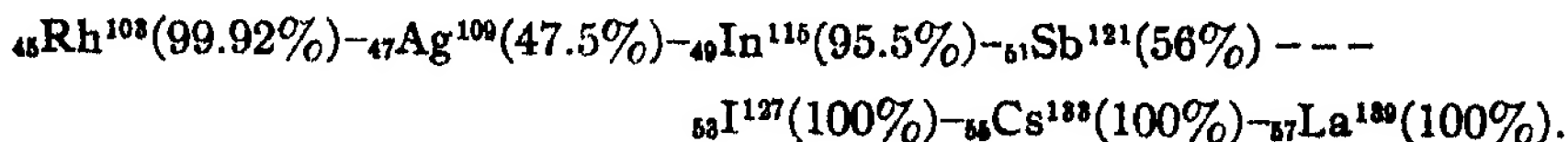
In this way series may be formed from  $r = 1$  to  $r = 22$ . For low values of  $r$  the number of members in such a series is rather small (one for  $r = 1, 2$ ; two for  $r = 3, 4, 5$ ; three for  $r = 6$ ; four for  $r = 7, 8, 9$ ). Then the series become longer, and are especially long for  $r = 18$  and  $r = 19$ ; these series contain 19 or 20 members, respectively, in uninterrupted sequence without any gap. For  $r = 20$  the number of members becomes ten, and then rapidly decreases towards the end of the scheme.

In the various series of table 2 many sequences can be noticed in which, at least in order of magnitude, the value of the relative abundance repeats itself several times. The following sequences seem especially noteworthy:  $r = 12$  from  $^{88}\text{Ni}$  to  $^{94}\text{Se}$ ;  $r = 16$  from  $^{96}\text{Pd}$  to  $^{100}\text{Sn}$ ;  $r = 18$  from  $^{96}\text{Pd}$  to  $^{100}\text{Sn}$  and from  $^{112}\text{Gd}$  to  $^{124}\text{W}$ ;  $r = 19$  from  $^{118}\text{Er}$  to  $^{130}\text{Hg}$  and from  $^{138}\text{Ra}$  to  $^{142}\text{U}$ .

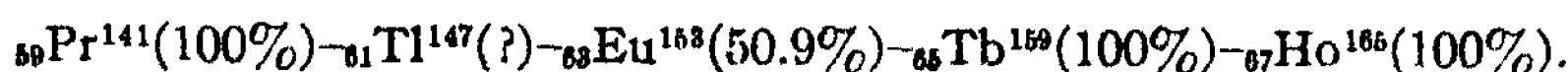
Similar regularities appear if we form a scheme of stable nuclei with odd charge and mass numbers in such a way that we add single protons to the nuclei of table 2, omitting all cases where the new nucleus is unstable or unknown. We find then, e.g., the following sequence for  $r = 8$ :



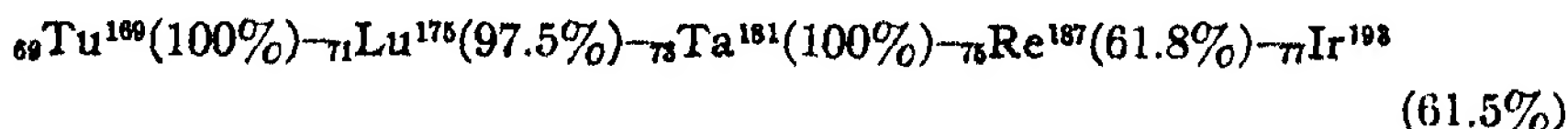
In the series  $r = 15$  values of the relative abundance of the order 1 and  $1/2$  are combined, namely



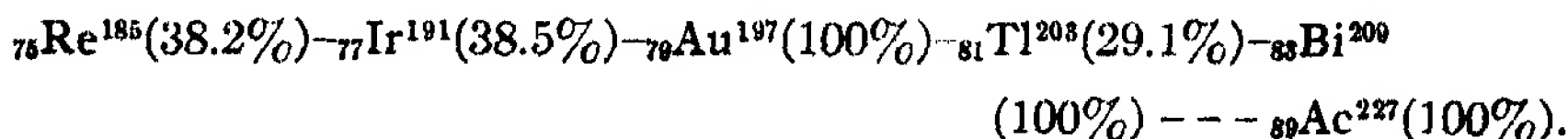
The same holds also for the series  $r = 17$ :



Values of the order 100% and 60% are combined in the series  $r = 18$ :



and values<sup>6</sup> of the orders 1 and  $1/3$  in the series  $r = 19$ :



Finally considering all stable nuclei (including those with odd charge and mass numbers) we observe a concentration of values of the relative abundance within a few rather narrow intervals. According to our present knowledge, 138 stable nuclei (among some 300) have relative abundances

TABLE 3

## DISTRIBUTION OF THE VALUES OF THE RELATIVE ABUNDANCE

50.6 to 50.9%	: ${}_{30}\text{Zn}^{64}(50.9)$ , ${}_{35}\text{Br}^{79}(50.6)$ , ${}_{63}\text{Eu}^{153}(50.9)$
26.8 to 27.3%	: ${}_{28}\text{Ni}^{60}(27.2)$ , ${}_{30}\text{Zn}^{68}(27.3)$ , ${}_{32}\text{Ge}^{73}(27.3)$ , ${}_{46}\text{Pd}^{106}(27.2)$ , ${}_{46}\text{Pd}^{108}(26.8)$ , ${}_{44}\text{X}^{132}(26.96)$
22.5 to 22.6%	: ${}_{46}\text{Pd}^{106}(22.6)$ , ${}_{50}\text{Sn}^{118}(22.5)$ , ${}_{60}\text{Nd}^{144}(22.6)$ , ${}_{64}\text{Gd}^{158}(22.6)$ , ${}_{64}\text{Gd}^{160}(22.6)$ , ${}_{74}\text{W}^{182}(22.6)$ , ${}_{82}\text{Pb}^{207}(22.64)$
16.5 to 18%	: ${}_{30}\text{Zn}^{68}(17.4)$ , ${}_{36}\text{Kr}^{84}(17.47)$ , ${}_{40}\text{Zr}^{94}(17)$ , ${}_{42}\text{Mo}^{98}(16.8)$ , ${}_{44}\text{Ru}^{104}(17)$ , ${}_{60}\text{Nd}^{146}(16.5)$ , ${}_{62}\text{Sm}^{147}(17)$ , ${}_{64}\text{Gd}^{167}(16.7)$ , ${}_{70}\text{Yb}^{173}(16.7)$ , ${}_{72}\text{Hf}^{179}(18)$ , ${}_{74}\text{W}^{183}(17.3)$ , ${}_{80}\text{Hg}^{199}(17.0)$
13.0 to 13.5%	: ${}_{46}\text{Pd}^{110}(13.5)$ , ${}_{48}\text{Cd}^{113}(13.0)$ , ${}_{60}\text{Nd}^{148}(13.0)$ , ${}_{76}\text{Os}^{190}(13.3)$ , ${}_{80}\text{Hg}^{201}(13.2)$
11.1 to 11.6%	: ${}_{12}\text{Mg}^{25}(11.6)$ , ${}_{13}\text{Mg}^{26}(11.1)$ , ${}_{36}\text{Kr}^{83}(11.53)$ , ${}_{36}\text{Kr}^{82}(11.53)$ , ${}_{40}\text{Zr}^{91}(11.5)$ , ${}_{56}\text{Ba}^{137}(11.32)$

between 9 and 91%. As may be seen from table 3, 39 of these 138 isotopes may be placed in six intervals which, altogether, cover a range of 4%. The intervals as recorded in table 3 lie slightly above the fractions  $1/2$ ,  $1/4$ ,  $1/6$ ,  $1/8$  and  $1/10$ . This may not be a mere coincidence, but perhaps caused by some quantum relations.

<sup>1</sup> Cf., e.g. W. D. Harkins, *Phys. Rev.*, **38**, 1270 (1931), and *Proc. Nat. Acad. Sci.*, **19**, 307 (1933); A. J. Dempster, *Proc. Amer. Phil. Soc.*, **76**, 491 (1936).

<sup>2</sup> The concept of the isotopic number was introduced into atomic physics by Harkins (1915).

<sup>3</sup> Unstable nuclei with even  $Z$  and  $A$  are  ${}_{2}\text{He}^6$  and  ${}_{36}\text{Kr}^{86}$ .

<sup>4</sup> The data used in this paper were taken from the survey by J. J. Livingood and G. T. Seaborg, *Rev. Mod. Phys.*, **12**, 30 (1940).

<sup>5</sup> In accordance with the rule that isobars nearly never differ by one in their charge numbers, table 1 contains only three nuclei with both symbols, *p* and *n*.

<sup>6</sup> In this connection it may be noted that the only known branching ratio in the natural radioactive series which is not very large or small, that of Th C, amounts to 65% or about  $\frac{2}{3}$ .

## MECHANISM OF LONG WAVE-LENGTH ABSORPTION OF THE CARBONYL GROUP

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Communicated March 6, 1940

Color in organic compounds is generally associated with systems of conjugated double bonds. Burawoy<sup>1</sup> has divided the spectra of such molecules into two classes, the strong *K* spectra and the weak *R* spectra. When both a *K* and an *R* spectrum are present for the same substance, the *R* spectrum lies at longer wave-lengths than the *K* spectrum.<sup>2</sup>

The *K* spectra of conjugated polyenes have been explained quantum-mechanically along the following lines.<sup>3, 4</sup> In ethylene and its derivatives the longest wave-length absorption region occurs near  $\lambda$  1800 and is characteristic of the C=C linkage. The electron configuration for the bonding electrons in the normal state *N* of this linkage may be written as follows in terms of MO's (i.e., molecular orbitals):

$$N: \dots (\mu)^2 (xx)^2. \quad (1)$$

Here  $(\mu)^2$  represents a pair of electrons forming the first or  $\sigma$  bond of the C=C double bond,  $(xx)^2$  a pair forming the second bond. The *xx* electrons are less firmly bound than the  $\mu$  electrons and are active in producing the longest wave-length absorption.

The MO's symbolized by  $\mu$  and *xx* have forms which may be approximated using simple combinations of AO's (i.e., atomic orbitals).<sup>5</sup> The symbol *x* represents a carbon  $2p_x$  AO, with its axis of symmetry in the *x* direction, perpendicular to the plane of the molecule. The symbol  $\mu$  represents a so-called trigonal AO, built up<sup>6</sup> from  $2s$  and  $2p_z$  carbon AO's and with its axis along the C=C bond direction (*z*-axis).

The lowest excited states *T* and *V* of ethylene and its derivatives are believed to belong to the following electron configuration:

$$T, V: \dots (\mu)^2 (xx)(x\bar{x}). \quad (2)$$

One of the two electrons originally present in the bonding  $\pi\pi$  MO has here been excited to the corresponding antibonding  $\pi\bar{\pi}$  MO.  $T$  is a triplet,  $V$  the corresponding singlet state. The transition  $N \rightarrow V$  comes near  $\lambda$  1800 and is very strong.<sup>6a</sup>

When other  $C=C$  groups are conjugated with the first  $C=C$  group the electrons of the  $\pi$  type do not remain localized within one group but occupy MO's covering the entire system of conjugated bonds, as has been shown by Hückel and others.<sup>7</sup> In approximating the forms of these non-localized  $\pi$  MO's, only the  $2p_x$  AO's of the carbon atoms need be considered. Simple calculations give the approximate forms and energies of as many MO's as there are carbon atoms in the conjugated system. In the normal state electron configuration each one of the MO's of lowest energy contains two electrons with opposite spin. The first excited state is obtained by removing an electron from the highest energy MO occupied in the normal state to the unoccupied MO of lowest energy. In general, as the number of conjugated groups increases the energy of the former of these MO's rises and that of the latter is lowered. Thus the energy difference between the normal state and the first excited state decreases with increasing conjugation and the first absorption region shifts toward longer wave-lengths. It also becomes more and more intense; reasons for this can be given.<sup>8</sup>

If instead of  $C=C$  groups alone certain other double-bonded groups, for example  $C=O$  or  $C=S$ , are present in the conjugated system, the same formal behavior for the energy of the MO's of the  $\pi$  type is present and a similar strong absorption, which shifts to longer wave-lengths with an increase in the number of conjugated bonds, is observed.

Here, however, another feature, characteristic of the new groups, appears in the form of a much weaker absorption region ( $R$  spectrum) coming at longer wave-lengths than the strong  $K$  type of absorption just described. In saturated aldehydes and ketones the maximum for this weak absorption is near  $\lambda$  2900.<sup>8</sup> In aromatic or conjugated compounds this absorption shifts toward or into the visible and can then be responsible for weak color, as, for example, in the quinones and in diacetyl or glyoxal.

The study of the long wave-length spectrum of formaldehyde has yielded a great deal of information concerning the  $R$  type of transition.<sup>9, 10, 11, 12</sup> The theoretical work of Herzberg and Teller,<sup>13</sup> combined with Herzberg and Franz's and Gradstein's study of the same spectrum in fluorescence, has strongly indicated that the electronic transition involved is a forbidden one. This interpretation was supported especially by the behavior of the  $A$  and  $\alpha$  bands in absorption and fluorescence.<sup>11, 14</sup>

Dieke and Kistiakowsky's analysis of the banded fine structure<sup>15</sup> gave further information. Possible interpretations of the transition in terms of specific electronic states were then given by one of the writers.<sup>16</sup> Two alternatives were favored, one corresponding to a certain allowed, the other



to a certain forbidden transition. Calculations made in the present work strongly support the second possibility, in agreement with the evidence mentioned above.<sup>16</sup>

The structure of the carbonyl group in its normal electronic state, so far as is essential for understanding the long wave-length spectrum, may be described by the following electron configuration:

$$N: \dots (zt)^2(xx)^2y_O^2. \quad (3)$$

The two pairs of electrons  $(zt)^2$  and  $(xx)^2$  form the  $C=O$  double bond, and correspond to  $(tt)^2(xx)^2$  of  $C=C$  (cf. equation (1)). The symbol  $y_O^2$  represents a pair of relatively loosely held non-bonding electrons in a  $2p_y$  AO of the oxygen atom. The letters  $x$ ,  $y$  and  $z$  denote  $2p$  AO's with axes respectively, along the direction perpendicular to the  $\angle C=O$  plane ( $x$ ), in the plane and perpendicular to the  $\angle C=O$  axis ( $y$ ), and along the  $\angle C=O$  axis ( $z$ ). The  $zt$  MO has a form which can be approximated by combining a  $2p_z$  oxygen AO and a  $t$  (trigonal) carbon AO. The  $xx$  can similarly be built from  $2p_x$  AO's of oxygen and carbon. These MO's are polarized in favor of the oxygen in accordance with the considerable negative charge on the latter.<sup>17</sup>

There seem to be only two important possibilities for the upper state of the  $\lambda$  2900 absorption region, namely, the singlet states of the following electron configurations:<sup>16</sup>

$$A: \dots (zt)^2(xx)^2y_O(x\bar{x}); \quad B: \dots (zt)^2(xx)^2y_O(z\bar{t}). \quad (4)$$

In  $A$  one  $y_O$  electron has been excited to the antibonding MO  $x\bar{x}$ , in  $B$  to the  $z\bar{t}$  antibonding MO.<sup>17</sup> Transitions from state  $N$  to either  $A$  or  $B$  should produce weakened  $C=O$  bonding. This is in harmony with the observed structure of the  $\lambda$  2900 region in formaldehyde.<sup>10, 12</sup>

The absorption transition from  $N \rightarrow A$  is forbidden, that from  $N \rightarrow B$  is allowed, by the electronic selection rules.<sup>15</sup> However, because of electronic-vibrational coupling, "forbidden" transitions are not really excluded in polyatomic molecules,<sup>18</sup> although they should be much weaker than similar allowed transitions.

Recently it has been found possible to make significant absolute intensity calculations for electronic transitions in molecules.<sup>18</sup> Comparison of calculated with observed intensities should often make it possible to decide between alternative interpretations of observed absorption regions.

For the present problem, calculations have been made for the transition  $N \rightarrow B$  (and for certain other transitions) using both the AO and the LCAO MO approximations.<sup>19</sup> These calculations indicate that the strength of the transition  $N \rightarrow B$  must be of the order of 50 times that ob-



served for the  $\lambda$  2900 region. This gives strong support to the identification of the  $\lambda$  2900 region with the forbidden transition  $N \rightarrow A$ .

On the other hand there exists in the spectra of aldehydes and ketones near  $\lambda$  1800 or  $\lambda$  1900 a region of absorption<sup>20, 21</sup> which (at least in acetone<sup>21</sup>) is known to have an intensity of the same order of magnitude as that calculated for  $N \rightarrow B$ . It seems probable that the  $\lambda$  1800 region is actually due to the transition  $N \rightarrow B$ . The assignment of the  $\lambda$  1800 absorption to a transition of the  $N \rightarrow V$  type, analogous to the intense longest wave-length absorption in ethylene, is excluded because the calculated intensity of such a transition<sup>3</sup> is much greater than that observed for the  $\lambda$  1800 region. Further, a theoretical estimate of the position of the  $V$  level of  $>C=O$  indicates that the  $N \rightarrow V$  transition should lie at shorter wave-lengths in saturated aldehydes and ketones than in ethylene and its derivatives.

One thus concludes that the relatively loosely bound pair of non-bonding electrons present in the oxygen atom of the carbonyl group, and not present in the ethylenic linkage, is responsible for the existence in saturated aldehydes and ketones of a characteristic absorption region of the carbonyl group near  $\lambda$  2900 and probably a second one near  $\lambda$  1800. The  $\lambda$  2900 region is at much longer wave-lengths than the first absorption of the ethylenic linkage.

If one or more  $C=O$  groups are substituted in place of  $C=C$  groups in a conjugated system of carbon atoms, one or more weak absorption regions appear at longer wave-lengths than when no  $C=O$  group is present. Thus in diacetyl (with two conjugated  $C=O$  groups) a weak absorption region is found near  $\lambda$  4000 (and a second near  $\lambda$  2900) while in butadiene (two conjugated  $C=C$  groups) the longest wave-length absorption is a strong region near  $\lambda$  2200. The evidence available indicates that this weak absorption shifts toward longer wave-lengths as the number of conjugated bonds increases.<sup>22</sup> Since conjugation lowers the energy of the first excited  $x\bar{x}$  type of MO while not greatly affecting that of the  $z\bar{t}$  type, this shift of the weak absorption toward longer wave-lengths constitutes strong evidence that the transition is of the  $N \rightarrow A$  and not of the  $N \rightarrow B$  type in conjugated (and also in saturated) aldehydes and ketones.

In carboxylic acids and esters the absorption corresponding to the  $\lambda$  2900 region of the  $>C=O$  group appears at shorter wave-lengths (near  $\lambda$  2100 in acetic acid). Estimates on the relative positions of the normal and lowest excited states for these molecules seem to explain this behavior satisfactorily.<sup>19</sup>

The chromophoric power of the  $>C=S$  group is explainable in the same way as that of  $>C=O$ ; the absorption is here shifted toward even longer wave-lengths because the non-bonding pair is more loosely bound in sulphur than in oxygen.

It appears then that the characteristics of Burawoy's  $R$  chromophores

can be explained in many cases in terms of transitions of the  $N \rightarrow A$  type discussed above.

<sup>1</sup> Burawoy, A., *Jour. Chem. Soc.*, 1177 (1939).

<sup>2</sup> Burawoy attributes the two types of spectra, respectively, to "K chromophores" and "R chromophores," which he believes cannot occur in the same molecule. He assumes the presence of a mixture of electronic isomers when a single substance gives both spectra. The quantum-mechanical discussion shows, however, that the same molecule can give both types of spectra.

<sup>3</sup> Mulliken, R. S., *Jour. Chem. Phys.*, 7, 22, 121, 364, 570 (1939).

<sup>4</sup> Sklar and Förster have also explained some of these spectra using the AO method of approximation (cf. Förster, T., *Zeit. Electrochemie*, 45, 548 (1939) for an excellent review and bibliography). Pauling, L. (these PROCEEDINGS 25, 577 (1939)) has recently applied the same method to the K spectra of dyes. The present paper employs the MO method of approximation.

<sup>5</sup> These (so-called LCAO) forms are of the type  $(A_1 \pm A_2)/(2 \pm 2S)^{1/2}$  where  $A_1$  and  $A_2$  stand for AO's of the two carbon atoms, and  $S = \int A_1 A_2 dv$ . In the present connection the  $A$  may stand for  $x$  or else for  $t$  (see text). The  $+$  signs apply for the bonding MO's  $xx$  and  $tt$ , the  $-$  signs for antibonding MO's  $x\bar{x}$  and  $t\bar{t}$ .

<sup>6</sup> Pauling, L., *The Nature of the Chemical Bond*, p. 88, footnote. (Cornell University Press (1939).)

<sup>6a</sup> Actually the longest wave-length region in  $C_7H_8$  and its derivatives contains also a Rydberg series transition superposed on the  $N \rightarrow V$  transition discussed here. R. S. Mulliken, *Jour. Chem. Phys.*, 7, 30 (1939); W. C. Price and W. T. Tutte, *Proc. Roy. Soc.*, 174A, 212 (1940). In the conjugated polyenes also there are Rydberg transitions, but probably all at shorter wave-lengths than the longest wave-length  $N \rightarrow V$  transition.

<sup>7</sup> See Hückel, Erich, *Zeit. Electrochemie*, 43, 752, 827 (1937); a general review discussing the electronic structure of unsaturated organic molecules.

<sup>8</sup> Eastwood, E., and Snow, C. P., *Proc. Roy. Soc.*, 149A, 434 (1935); Scheibe, G., and Frömel, W., *Eucken-Wolf Hand- und Jahrbuch der chemischen Physik*, 9, Part IV (1936), especially from p. 164 on; *International Critical Tables*, Vol. 5, and Landolt-Börnstein, *Physikalisch-Chemische Tabellen*. These references give absorption curves and further references relating to these and other molecules.

<sup>9</sup> Henri, V., and Schou, S. A., *Zeit. Phys.*, 49, 774 (1928).

<sup>10</sup> Herzberg, G., and Franz, K., *Ibid.*, 76, 720 (1932).

<sup>11</sup> Gradstein, S., *Zeit. phys. Chem.*, B22, 384 (1933).

<sup>12</sup> Dieke, G. H., and Kistiakowsky, G. B., *Proc. Nat. Acad. Sci.*, 18, 367 (1932); *Phys. Rev.*, 45, 4 (1934).

<sup>13</sup> Herzberg, G., and Teller, E., *Zeit. phys. Chem.*, B21, 410 (1933).

<sup>14</sup> Similar features and a similar absorption strength are found in the  $\lambda$  2600 region of benzene, recently established as a forbidden transition. (Sponer, H.; Nordheim, G., Sklar, A. L., and Teller, E., *Jour. Chem. Phys.*, 7, 207 (1939).)

<sup>15</sup> Mulliken, R. S., *Ibid.*, 3, 564 (1935).

<sup>16</sup> Also Scheibe, G., and Frömel, W. (see ref. 6, p. 169) point out that the intensity of this transition in saturated aldehydes and ketones is sensitive to changes in the substituents in harmony with what is expected for a transition of the forbidden type.

<sup>17</sup> The LCAO forms of the  $st$ ,  $xx$ ,  $x\bar{x}$ ,  $s\bar{t}$  MO's here are similar to those for the analogous MO's of  $C=C$  given in ref. 5. Now, however,  $A_1$  refers to  $2p_z$  or  $2p_x$  of oxygen. Further, the coefficient of  $A_1$  in the LCAO form is, for the bonding MO's, now somewhat larger than that of  $A_2$ . This corresponds to the existence of net negative charge on the oxygen. For the antibonding MO's  $x\bar{x}$  and  $s\bar{t}$  the polarity is reversed.

<sup>18</sup> Reference 3 and following papers, especially *Jour. Chem. Phys.*, March, May, 1940.

<sup>19</sup> These calculations will be reported in detail elsewhere by one of the authors.

<sup>20</sup> Scheibe, G., Povenz, F., and Linström, C. F., *Zeit. phys. Chem.*, **B20**, 283 (1933); Noyes, W. A., Jr., Duncan, A. B. F., and Manning, Winston, *Jour. Chem. Phys.*, **2**, 717 (1934); Duncan, A. B. F., Ellis, V., and Noyes, W. A., *Jour. Amer. Chem. Soc.*, **58**, 1454 (1936); Price, W. C., *Jour. Chem. Phys.*, **3**, 256 (1935).

<sup>21</sup> Ley, H., and Arends, B., *Zeit. phys. Chem.*, **B12**, 131 (1931).

<sup>22</sup> See Ref. 8; particularly Scheibe and Frömel.



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*SIMULTANEOUS MEASURES OF HUMAN RELATIONS AND  
EMOTIONAL ACTIVITY*

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The quantitative description of human relations is based upon the measurement of the interaction of individuals. This description involves the continuous measurement of the durations of actions and inactions of observed persons as one acts and another is silent, and also includes all instances of interruptions (when both act) and of failures to respond (when both are silent). The methods of measurement and the discriminations used have been described elsewhere,<sup>1,2</sup> but it must be repeated here that in these measurements no attempt is made to evaluate the subjective experience of the individuals by interpreting the "meaning" of the words and gestures making up the actions. It is believed that a satisfactory description of human relations (the adjustment of individuals) is to be obtained from the quantitative analysis of the measured values of the actions and inactions in interaction in terms of the changes in synchronization of the interacting individuals. Since the actions of organisms, whether "spontaneous" or in response to stimuli, clearly involve modifications of the internal environment, we should expect that the "emotions" which play such a prominent part in our subjective experience of relations with other people would be directly associated with the interaction. Hence, simultaneous measures of human relations and emotional activity ought to provide a test of this hypothesis.

Estimates of the degree of adjustment of individuals to one another and of the associated emotions have always played a large part in the accounts of human behavior by social and psychological investigators. Ordinarily, these accounts have had a frankly subjective character, but in some instances various measures of physiological response have been utilized. These have included (to name only those most frequently used) the measurement of the electrical activity of the brain,<sup>3</sup> the electro-galvanic response,<sup>4</sup> the heart rate,<sup>5</sup> the respiration rate,<sup>6</sup> blood pressure,<sup>6</sup> muscular tension.<sup>7</sup>

In each case, however, the stimulus eliciting the measured response has always been qualitative—the presentation to the subject of selected symbols supposed to have “emotional” significance. Not only is there no precise evidence of the connection of any given symbol with the behavior even of a single individual, but the conditions of experiment, when involving interaction, introduce further difficulties if interaction can be shown to produce alterations in emotional response. In any case, the use of operations to obtain a measurement of the magnitude of a physiological response is liable to conceal the fact that the nature of the stimulus is such that we are not comparing our measured values with measured values (for the stimulus) of known dimensions, but rather with qualitatively discrete phenomena whose only quantification derives from the measured response.

In laboratory studies of emotion by physiologists, where the purpose of the experiment is to describe the changes in the organism associated with the overt manifestation of emotion, there is more evidence, from the nature of the experiments, of the relationship between interaction and emotional activity. In these studies the laboratory animal, usually a cat, is tied to an animal board and then put into a state of rage or fear. This is often done by taking advantage of the previous conditioning of the animal and, for example, bringing in a dog who barks at and tries to attack the cat. The cat responds to the succession of stimuli provided by the dog's actions, and the overt activities of the skeletal muscles of the cat in interaction are associated with changes in the internal environment produced by the activation of the autonomic nervous system, measured by changes in heart rate, blood sugar and adrenalin concentration, and so on.<sup>8,9</sup> These manifestations of emotional activity involving changes in the neuro-humoral system are regarded as the response of the organism to disturbances of its equilibrium, or of what Cannon calls homeostasis.<sup>10</sup> These disturbances may be divided into stimuli of “high” intensity and stimuli which are repeated a number of times. In each of these divisions the factor of the durations of the periods of the stimulus and the frequency of presentation of the stimulus are implicit in the experimental situation, but are ordinarily unspecified.

The use of the electroencephalograph as a measure of emotional activity developed from the observations of Hoagland, *et al.*, on schizophrenics undergoing insulin treatment.<sup>11</sup> In studying the brain wave records marked variations in the so-called delta waves were noticed. It was found that an estimate of the amount of delta activity provided a useful and quantitative account of changes in the patient's condition, not only during hypoglycemia but also from day to day. In a number of cases studied, changes in the state of the patient as reported from the clinical history were correlated with changes in the values of the delta index (the measure contrived by Hoagland<sup>11</sup> to quantify the delta waves). In the course of these



observations, it was also observed that marked increases in the delta index were associated with emotional crises, and a series of observations was then made to test the hypothesis that delta wave activity is directly associated with emotion. The technique used was the so-called psychic probe, in which material taken from the patient's case history is repeated in the form of questions to the patient. These questions referred to situations apparently having strong emotional connotations. Between each question, there was a period of silence. Marked increases in the delta index were correlated with the periods of questioning.<sup>3</sup>

It was then suggested that the principal source of the delta waves was the hypothalamus, since the work of Cannon, Bard and others has demonstrated that the hypothalamus is the controlling mechanism in the neuro-humoral system (responsible for emotion).<sup>9,12</sup> Using a method developed by Grinker,<sup>13</sup> simultaneous records of psychic probe observations were made from the occipital region and from a region near the hypothalamus. Not only does the evidence indicate the strong predominance of the delta waves in the hypothalamic record, but Hoagland, *et al.*, were able to show that the delta waves at the hypothalamic lead preceded the corresponding delta waves at the cortical lead by an average of about four milliseconds.<sup>14</sup>

During the summer of 1939, Dr. Hoagland kindly brought to our laboratory his electroencephalograph, a new instrument built by Grass, with which three simultaneous records could be taken. After preliminary experimentation with the subjects sitting upright, it was decided, in order to avoid any possibility of muscle artefacts, to have the subjects in the standard position for taking electroencephalograms, namely, lying down on cots with the eyes closed. Twelve conversations between pairs of individuals were then recorded, each lasting between fifteen and twenty minutes. After a preliminary control period to obtain the brain wave record in the resting position, the subjects were instructed to talk, and the beginning of the interaction marked on the tape. At the same time, Mr. Lin recorded the beginning of the action on the recording apparatus. When the interaction ended, another control period was taken and tests were also made to eliminate the possibility that any of the muscle activities in interaction produced muscle artefacts in the record. The analysis of the records has brought out clear-cut relationships between the interaction records and brain waves not only for the delta waves but also for the alpha waves (Berger rhythm).

On each electroencephalogram tape, the values of the durations of actions and inactions for each person were plotted in the order of occurrence. This included the double actions (interruptions) and double silences (failures to respond). We thus obtain concurrent records of the electrical activity of the brain and of the interactions of the individuals.

After the record of the resting period taken as control at the beginning of

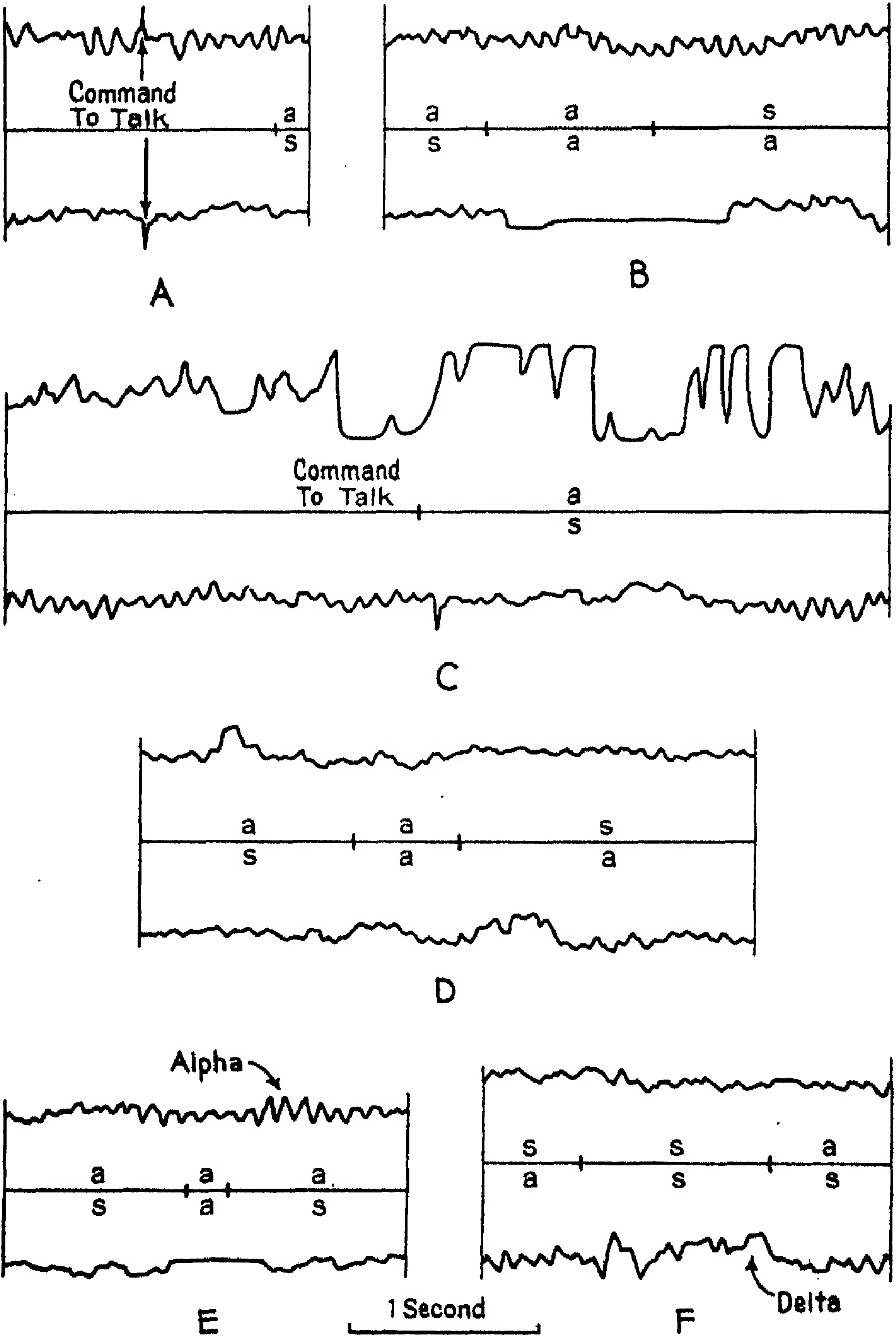


FIGURE 1

the observation was completed, the operator of the electroencephalograph gave the command to start talking. In all records, a characteristic "startle spike" occurred of which an example is given in *A* of figure 1. In this figure, the upper curve represents the brain wave of one individual, the lower curve the simultaneous curve of the other individual with whom he interacts. The center line has plotted on it the actions and silences of the two persons, the upper being the action-silence record of the individual whose brain wave record is given above, the lower sequence belonging to the individual having the lower brain wave. The vertical lines cutting the center line indicate the point at which a change of action takes place. The approximately similar position on the records for both individuals, and the fact that it is found in all records at the point marked in pencil on the tape where the command to begin was given, indicates that this may be regarded as an instance of a "startle pattern" in electrical activity.

(It should be explained, for the benefit of those not familiar with electroencephalogram records, that alpha waves have a frequency of about ten a second and trace out fairly regular sine curves. Delta waves are slower in frequency, commonly about four a second, although they may approach about one second in their duration. Usually the term delta wave refers to all waves slower than alpha waves. In figure 1, where the scale is 3.0 centimeters to the second, examples of alpha and delta waves are pointed out.)

In double silences and double actions predominantly, and ordinarily at the beginning and ending of actions and silences, marked changes in the electroencephalograph were found. The kind of change seemed to depend upon the amount of alpha activity that the individual was manifesting on the given day. Although in a rough approximation, individuals may be classified in terms of the proportion of alpha activity on any given meter of tape during the resting period, the variation for the individual on different days or even within the same day is so great that one cannot properly speak of individuals having a high or low alpha predominance since differences of twenty-five to thirty per cent or higher occur on different days. When an individual, who at the time of observation has a per cent time alpha frequency of about fifty per cent, interrupts or is interrupted or when a double silence occurs or when he begins or ends an action or silence, there occurs a characteristic damping of the alpha waves, an example of which is to be seen in the top record in *E* of figure 1 as well as in the bottom record of *C* after the startle spike. Less frequently, besides the damping of alphas a marked delta wave will appear. In individuals who have a low per cent time alpha, and this includes individuals who on other days manifest a high alpha frequency (above 50%), there occurs a characteristic flattening of the low irregular waves into what is almost a straight line which may often be maintained to one side of the abscissa. Examples of this phenomenon are given in the lower records of *B* and *E* in figure 1. (It is of inter-

est to point out that these two records as well as the bottom record of *C* where there is much alpha activity are taken from the same individual.) In other cases, just as for individuals having higher alpha frequencies, clear-cut delta waves appear. Two examples of the appearance of delta waves are given in the bottom records of *D* and *F* in figure 1, the first for a double action and the second for a double silence. The waves associated with a change from an action to a silence, or vice versa, are illustrated by the upper record of *D* in figure 1 as well as in the extraordinary activity in the upper record of *C*. This latter record also illustrates clearly a further uniformity which we have observed. There is always an increase in delta activity (recorded by the delta index) at the beginning of interaction. In the upper record of *C* in figure 1 the subject, in a highly excited state, had a delta index of 30 during the resting period. This jumped at the beginning of interaction, marked "talk," to an estimated 90, where, as is clear from the record, the ink writer was forced continuously to the limit of its freedom of swing. One further observation is illustrated in the lower record of *C* in figure 1. At the beginning of an action or silence, the alpha waves damp out in individuals. (It must be remembered that all individuals had their eyes closed so that visual activity was eliminated as a factor in the fading of the alphas.) In long actions and in long silences, after a period of fading, the alphas came in again. In the record just referred to, at the end of the strip shown during all of which time the individual was silent, the alphas reappear on the tape. This also happens at longer intervals for cases in which the individual is acting rather than being silent.

Not only did changes of the sort described occur in association with double actions and silences and the beginning and ending of actions and silences, but changing values of the interaction rates (to be described elsewhere) were associated with changes in the frequency of delta waves. An individual arrived at the observation room and manifested a given value of the delta index in the resting period. Throughout the interaction these values changed accompanying changes in the interaction rates. The repetition of double actions, for example, and their associated delta waves, were followed by trains of delta waves which gradually disappeared, unless further stimulation took place. Although these changes may be roughly estimated by the use of the delta index, no precise method is available for quantification, since it is impossible in all cases to know how the irregular activity in the alpha waves or the long swells of over a second are related to the delta activity. Moreover, the delta index is not convenient when used in association with measures of interaction because of the difference in the operations used in the two measurements.

Characteristic of the interaction record of any individual is the cyclical fluctuation in the duration of the actions and silences. The quantitative analysis of interaction is based upon the description of these fluctuations

and the variations that occur as a result of double actions and double silences and the changes in the phase relations of the action and silence values. The measurements are all taken in durations of seconds, while the delta index is taken in terms of the excess distance traveled by a map measure over the long waves (more than 0.10 in period) over the meter distance (33.3 seconds). Since actions and silences vary in duration, the arbitrary application of the meter provides measures not based on homogeneous values of the actions and silences whose relationships provide the basis for the analysis. This matter and a preliminary solution will be discussed in a future publication. Nevertheless, it should be pointed out that increases in delta activity, as one might expect from the discussion in this paper, measured in terms of the delta index, vary with the double actions and double silences and the relationship of the actions to the silences.

The precise relationship of brain waves and interaction can only be demonstrated when parameters derived from the quantitative analysis of each type of measurement can be shown to be functionally related. Nevertheless, the evidence indicates that changes in the electroencephalogram—damping of alphas for high per cent time alphas, flattening in low per cent time alphas, as well as the occurrence of delta waves—are associated with double actions and double silences and the beginning and ending of actions and silences. The occurrence of delta waves at these points, and the appearance of levels of delta activity following changes in interaction, suggest that "emotion," if delta activity is a satisfactory measure of these changes, is produced by changing values of the interaction of individuals. The relationship of delta activity with the neuro-humoral system is not only indicated by experiments with a hypothalamic lead and the studies on the hypothalamus, but also by simultaneous measures of changes in heart rate and blood sugar concentration and the delta index.<sup>8</sup> It therefore seems probable that changes in the bodily states (as the physiological evidence indicates) must be regarded as activated by the stimulus-response situation set up in interaction. In any case, care must be taken in studies of emotion to control the interaction of the individuals taking part in the experiments.

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<sup>3</sup> Hoagland, H., Cameron, D. E., Rubin, M. A., *Jour. Gen. Psychol.*, 19, 227-245 (1938).

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<sup>5</sup> Boas, E. P., and Goldschmidt, E. F., *The Heart Rate*. Baltimore: Thomas, 1932, pp. xi + 166.

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<sup>7</sup> Luria, A. B., *The Nature of Human Conflicts*. New York: Liveright, 1932, pp. 431.

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<sup>13</sup> Grinker, R. R., *Science*, **87**, 73-4 (1938).

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## GALACTIC AND EXTRAGALACTIC STUDIES, VII. MAGNITUDES OF FORTY CEPHEIDS IN THE LARGE MAGELLANIC CLOUD

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1. *Introduction.*—The Large Magellanic Cloud, with more than thirteen hundred known variable stars, offers an excellent body of homogeneous material for the study of light-curve characteristics of classical Cepheid variables. Of particular interest are the problems of the correlation of period with form of light curve and the variation of amplitude with period and absolute luminosity. The interpretation of stellar atmospheres will obviously be assisted by any reliable evidence on relations between period length and the details of light variations that result from pulsations in the interiors or in the surface layers of stars.

On the basis of data obtained for galactic Cepheids, a number of observers, especially Ludendorff,<sup>1</sup> Hertzsprung,<sup>2</sup> Lundmark<sup>3</sup> and Gaposchkin,<sup>4</sup> have discussed the appearance and location on the light curves of abrupt changes in the rate of increase or decrease of light—that is, the occurrence of humps or secondary maxima and minima. Although the number of adequate light curves has been small, and the observations not altogether homogeneous, the indication of variation of form of curve with period has been fairly consistent, at least over a considerable range of period.

We turn to the wealth of material in the Large Magellanic Cloud for a further examination of the question for two reasons in particular:

(a) We have here a uniform system of apparent magnitudes, suitable for many Cepheids that are near together and show a wide variety of periods.

(b) A large number of plates made with the 24-inch Bruce refractor are now available.



The faintness of the Cepheids in the Large Cloud, and the various photometric confusions arising from the Cloud's background of rich star fields and bright and dark nebulosity, make it difficult to obtain accurate magnitude estimates. The results in the present study are therefore not as precise as we had hoped, and our light curves must be considered as a contribution to the study of a question not yet wholly settled.

2. *The Observations.*—Space cannot be taken to list and describe the two hundred and ninety-seven Bruce plates that are the basis of the investigation, nor is it possible to publish the individual magnitudes, eighty-two hundred in number, of the forty variables measured. The observations can, however, be made available to any investigator desiring to use them. Mean magnitudes will be published soon in *Harvard Annals*, 90, No. 10 (1940).

The plates used are predominantly in the homogeneous series made in the decade of 1926–1936 at Bloemfontein under the supervision of Dr. J. S. Paraskevopoulos; but some date back to 1896. The early plates were included in order that the periods might be checked and improved, and evidence sought for changes of period. By decades, the numbers of plates employed are: 1896–1906, 31; 1906–1916, 7; 1916–1926, 41; 1926–1936, 195; since 1936, 23.

In table 1 the stars are listed in order of increasing period. The positions can be obtained, if desired, from the coördinates published in *Harvard Annals*, 90, No. 1 (1933). Periods have been carefully examined by Miss McKibben, and those of the thirty-four variables previously published in *Harvard Bulletin* 905 (1937) have been revised or determined to a higher degree of accuracy. The only revisions necessary, however, were in the last decimal places of the values as published; some of the periods had been determined only approximately. The corrections to period are given in the third column of table 1. For six of the stars the periods had not been determined heretofore.

The magnitudes given in columns five, six, seven and eight are based on smooth curves drawn through normal points of five observations each. The magnitude estimates were based on five sequences that have been thoroughly intercompared and standardized.<sup>5</sup> For one star, H. V. 2809, the distance from the nearest sequence may have introduced a systematic error of two-tenths of a magnitude, but no correction has been made for this possible error. The number of observations in the ninth column varies because of varied plate-centering and also because plate quality has occasionally made advisable the omission of measures on faint stars.

In the last column of table 1, the description of the field is intended to indicate possible causes of unusual scattering in the observations, unusual ranges of variation or faintness produced by obscuring nebulosity. The letters have the following meanings:

<i>p</i> , poor star field	<i>e</i> , on edge of obscuration
<i>r</i> , rich star field	<i>o</i> , in obscured area
<i>i</i> , average star field	<i>o'</i> , very dense obscuration
<i>c</i> , clear of nebulosity	<i>d</i> , faint companion star in contact
<i>d</i> / doubling distorts image and decreases range	

3. *The Period-Luminosity Relation*.—From columns four and eight of table 1 we find the period-luminosity relation as defined by this limited but homogeneous collection of material (Fig. 1). The line through the plotted points is

$$\log P = 7.32 - 0.42\dot{m},$$

where  $\dot{m}$  is the median apparent photographic magnitude; the relation holds in the interval between periods of 2.5 and 50 days.

The dispersion about the mean period-luminosity curve can be attributed to various causes—thickness of the Cloud, intrinsic dispersion, absorption, doubling, photometric measuring errors. This scattering, and the period-luminosity relation in general, will be discussed in some detail in another paper, involving a treatment of the periods and median magnitudes of more than four hundred classical Cepheids.

4. *Amplitudes, Periods and Median Magnitudes*.—In figure 2*a* the relation of period to range of variation is shown, and in figure 2*b* the relation of range to median apparent magnitude. Except for the small but unknown correction for thickness of the Cloud, this second plot could be taken as range against magnitude on the absolute scale.

Again there are outstanding residuals. The three stars H. V. 5954, H. V. 2432 and H. V. 999 (circles in both diagrams) have small observed ranges which, according to the field description in table 1, are caused by doubling—the inclusion of a companion star in the measures. There is some evidence (Fig. 1) that these stars also suffer strong absorption, for otherwise their median magnitudes should be brighter than shown by the circles on the plot of the period-luminosity relation. The three deviating low-weight points have been allowed for in drawing the mean curves, which are given by

$$\begin{aligned}\log P &= 1.32 A - 0.68 \\ \dot{m} &= -2.63 A + 18.56\end{aligned}$$

where  $A$  is the amplitude in magnitudes, and the relations are applicable only in the interval from 2.5 to 20 days in period and from 14.7 to 16.5 in median magnitude.

That the amplitude of a Cepheid generally varies with the period has long been known,<sup>6</sup> but a quantitative derivation of the dependence has been hindered heretofore by the difficulties of reducing the scattered light

curves to a uniform system. The smallest ranges shown in our material may be produced by companion stars, but some galactic Cepheids (Polaris, for example) exhibit small ranges for which this explanation is less acceptable (see Hertzsprung's discussion, *loc. cit.*).

5. *Period and Form of Light Curve.*—Light curves of the forty Cepheids will be published with the observations in the *Harvard Annals*. Here it will suffice to summarize our somewhat indefinite conclusions concerning the relations of light-curve forms to period lengths.

(a) The most distinct relation found in this material is the tendency of amplitude to vary with period (and magnitude), illustrated in figure 2.

(b) Although there is no unambiguous dependence of shape of curve on period, the results of Ludendorff and Hertzsprung are roughly confirmed. Only three curves show distinctly double maxima, and they are the three with periods between 9.5 and 11.3 days. Less distinct secondary maxima

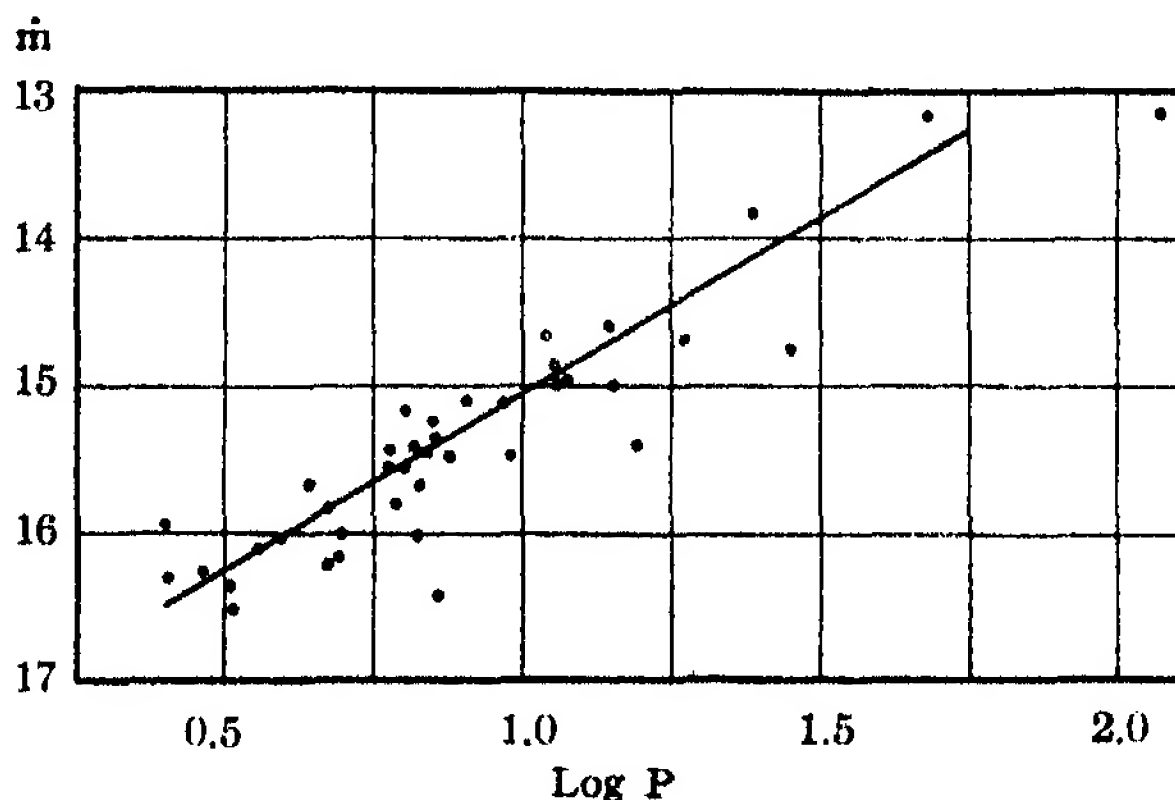


FIGURE 1

Period-luminosity relation.

or humps on the descending branch of the light curves appear throughout the series, but are most conspicuous for periods just shorter than those of the double-maximum stars. In his discussion of thirty-seven galactic Cepheids Hertzsprung finds the nearest approach to double maxima between 7.6 and 8.4 days, where our light curves are nearly smooth. This shift of the phenomenon to longer periods in the Large Cloud may be significant in Cepheid theory, if further work definitely establishes the difference.

(c) As also noted by Ludendorff and Hertzsprung, the light curves approach a symmetrical form when the periods are between 9.5 and 12.0 days; but the steep ascending branches are rather widely scattered throughout the whole range of periods, with highly asymmetrical light curves prevailing for all periods between 15 and 30 days.

(d) If we designate by  $a$  the interval of time between maximum and the moment of median magnitude on the ascending branch, and by  $b$  the in-

TABLE 1

## FORTY VARIABLES IN THE LARGE MAGELLANIC CLOUD

H. V. NO.	PERIOD IN DAYS	$\Delta P$	LOG. PERIOD	PHOTOGRAPHIC MAGNITUDES				NO. OF OBS.	FIELD DESCR.
				MAX.	MIN.	RANGE	MED.		
2809	2.505611	-0.000011	0.399	15.36	16.53	1.17	15.94	184	<i>i, c</i>
2344	2.524408		0.402	15.96	16.62	0.66	16.29	189	<i>i, e?</i>
2796	2.89383	-0.00003	0.461	15.79	16.72	0.93	16.26	179	<i>i, e?</i>
2368	3.2234		0.508	15.92	16.81	0.89	16.36	192	<i>i, e?</i>
2361	3.250875	+0.001125	0.512	16.03	16.98	0.95	16.51	178	<i>i, e</i>
2472	3.606651		0.557	15.71	16.49	0.78	16.10	189	<i>p, c?</i>
2795	3.913320	+0.000080	0.593	15.48	16.58	1.10	16.03	187	<i>i, e?</i>
2788	4.355729	+0.000271	0.639	15.15	16.20	1.05	15.67	192	<i>i, e?</i>
2334	4.691411		0.671	15.75	16.66	0.91	16.21	191	<i>i, e?</i>
2826	4.709949	+0.000151	0.673	15.39	16.28	0.89	15.83	193	<i>p, c</i>
951	4.8806486		0.688	15.66	16.67	1.01	16.16	186	<i>p, c</i>
2861	4.93822		0.694	15.50	16.49	0.99	16.00	198	<i>i, c?</i>
2727	5.94997	+0.00063	0.775	14.95	16.16	1.21	15.55	237	<i>p, o</i>
2619	5.97746	+0.00014	0.777	14.72	16.10	1.38	15.41	214	<i>p, o, d?</i>
5954	6.13595	-0.00595	0.788	15.51	16.07	0.56	15.79	237	<i>p, o, d</i>
2773	6.349005	-0.000005	0.803	15.03	16.08	1.05	15.55	232	<i>i, c?</i>
2536	6.37075	+0.00025	0.804	14.51	15.82	1.31	15.17	238	<i>i, e?</i>
2685	6.5415	0.0000	0.816	14.86	15.94	1.08	15.40	237	<i>i, e, d?</i>
2790	6.60615	+0.00015	0.820	15.56	16.46	0.90	16.01	185	<i>r, e</i>
2358	6.67690	-0.00690	0.825	15.32	16.02	0.70	15.67	218	<i>i, e, d?</i>
2337	6.86365	-0.00065	0.837	14.85	16.02	1.17	15.44	207	<i>i, e?, d?</i>
935	7.0674	-0.0003	0.849	14.63	15.82	1.19	15.23	249	<i>i, c?</i>
2491	7.1327	+0.0003	0.853	14.80	15.92	1.12	15.36	218	<i>p, c</i>
2752	7.1943	-0.0043	0.857	15.78	17.04	1.26	16.41	191	<i>p, e</i>
927	7.5282	-0.0002	0.877	14.87	16.07	1.20	15.47	199	<i>p, o!</i>
2722	8.02652	-0.00052	0.905	14.47	15.73	1.26	15.10	220	<i>i, e?</i>
971	9.29619	+0.00581	0.968	14.56	15.66	1.10	15.11	200	<i>i, e?</i>
952	9.5715	+0.0005	0.981	14.95	16.02	1.07	15.48	197	<i>i, e?</i>
2432	10.9245	+0.0005	1.038	14.35	14.97	0.62	14.66	201	<i>p, c, d</i>
999	11.2353	+0.0247	1.051	14.56	15.15	0.59	14.86	201	<i>i, c?, d!</i>
2787	11.442	-0.042	1.058	14.48	15.51	1.03	14.99	200	<i>i, e?</i>
905	11.85787	+0.00013	1.074	14.26	15.66	1.40	14.96	235	<i>r, o, d?</i>
2463	13.95235	-0.00235	1.145	13.80	15.41	1.61	14.60	201	<i>p, e?</i>
1006	14.21141	+0.00059	1.153	14.25	15.75	1.50	15.00	197	<i>r, e</i>
933	15.5441	+0.0009	1.192	14.66	16.15	1.49	15.40	198	<i>p, e</i>
1005	18.709759	+0.000241	1.272	13.96	15.42	1.46	14.69	199	<i>i, e?</i>
1003	24.388661	+0.006339	1.387	13.22	14.44	1.22	13.83	201	<i>i, c?</i>
934	28.18648	-0.00648	1.450	14.00	15.50	1.50	14.75	220	<i>i, c?</i>
953	47.82607	-0.12607	1.680	12.50	13.87	1.37	13.18	201	<i>i, c?</i>
2447	118.6394	-0.1394	2.074	12.65	13.66	1.01	13.16	201	<i>p, c?</i>

terval between maximum and the moment of median magnitude on the descending branch, the ratio  $a/b$  is a measure of the asymmetry of the

light curve at maximum. In groups of five stars, in order of increasing period, we have:

Mean $P$	2 <sup>d</sup> .88	4 <sup>d</sup> .26	5 <sup>d</sup> .58	6 <sup>d</sup> .51	7 <sup>d</sup> .16	9 <sup>d</sup> .81	13 <sup>d</sup> .40	47 <sup>d</sup> .55
Mean $a/b$	0.49	0.37	0.50	0.39	0.35	0.70	0.64	0.38
Av. dev.	0.09	0.05	0.10	0.07	0.08	0.34	0.19	0.13

Selecting from the sixth and seventh groups the five stars with periods between 9.5 and 12.0 days, the values are 11<sup>d</sup>.01, 0.88 and 0.20, showing that in this interval of period the asymmetry at maximum is least. If we

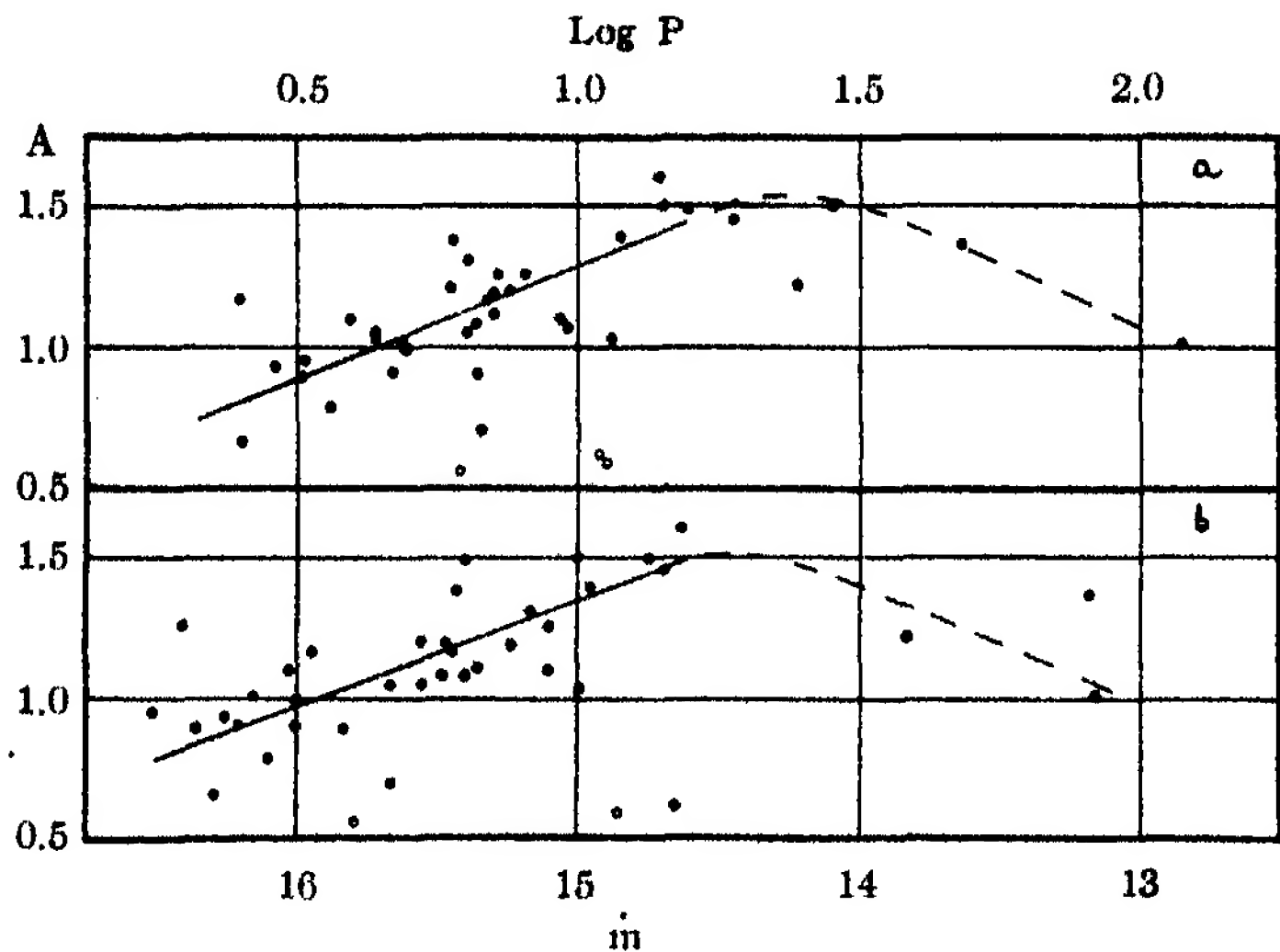


FIGURE 2  
Amplitude against (a) logarithm of the period and (b) median photographic magnitude.

omit the five stars H. V. 953, 2344, 2447, 2752 and 2796, for which the scatter of the individual observations is the greatest, and for which the form of light curve may therefore be least securely determined, we have:

Mean $P$	3 <sup>d</sup> .30	4 <sup>d</sup> .72	6 <sup>d</sup> .16	6 <sup>d</sup> .75	8 <sup>d</sup> .31	11 <sup>d</sup> .88	20 <sup>d</sup> .21
Mean $a/b$	0.43	0.40	0.47	0.36	0.41	0.92	0.34
Av. dev.	0.07	0.07	0.12	0.03	0.09	0.17	0.08

(e) Evidence for a decrease, with increasing period, in the separation of maximum and hump, suggested by Ludendorff and Lundmark, is not strong in this material. At the best the shifting is a tendency, not an invariable rule; and it is not likely that by increasing the accuracy of the individual observations the exceptions will be completely removed.

6. *Summary.*—With what appears to be a homogeneous system of magnitude standards, the light curves of forty classical Cepheids in the Large Magellanic Cloud have been determined from estimates made on plates with the 24-inch Bruce refractor. The whole range of known periods, from 2.5 to 118 days, is represented, but special attention has been paid to stars with periods between four and twenty days.

Light elements have been found for six of the variables, and the earlier values of the periods for the other thirty-four have been revised.

Abnormally small amplitudes for three variables are attributed to the influence of close companion stars.

Throughout intervals of median magnitude and period that include most of the classical Cepheids in the Large Cloud, the amplitude increases with period and therefore with absolute magnitude.

The variation of the form of the mean light curve with the period is summarized in Section 5.

<sup>1</sup> Ludendorff, *Astr. Nach.*, 209, 217 (1919), and *Sitzb. Preuss. Akad. Wiss.*, No. 5 (1929).

<sup>2</sup> Hertzsprung, *B. A. N.*, 2, 83 (1924), and 3, 115 (1926); cf. Sohn, *B. A. N.*, 3, 204 (1926).

<sup>3</sup> Lundmark, *Lund Obs. Circ.*, No. 5 (1932).

<sup>4</sup> Gaposchkin, these PROCEEDINGS, 24, 1 (1938).

<sup>5</sup> For a discussion of the magnitude system, see J. Mohr, *Harv. Ann.*, 105, No. 11 (1937), and Shapley, *Harv. Circ.* 255 (1924).

<sup>6</sup> For example, see Lundmark's discussion, *Lund Medd.*, Ser. I, No. 128 (1931).

## FURTHER REMARKS ON THE COSMOLOGICAL TIME SCALE

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Recent investigations on clusters of nebulae with the 18-inch Schmidt telescope on Palomar Mountain suggest that many large clusters of nebulae such as the Coma cluster represent *statistically stationary* configurations.<sup>1</sup> Calculations show that, if one starts from originally random distributions of nebulae, the formation of stationary large clusters requires periods of time exceeding  $10^{18}$  years.<sup>1</sup> Consequently, if the process of clustering of nebulae has actually reached a stationary state, it follows that the short-time scale demanded by the hypothesis of an expanding universe is untenable.

Objections to the considerations just sketched have recently been advanced by M. S. Vallarta<sup>2</sup> on the ground that G. Lemaitre's theory<sup>3</sup> of the formation of clusters in an expanding universe was not taken into account.



The following lines are intended to show that Lemaitre's considerations in no way affect the calculations which I have advanced<sup>1</sup> in order to demonstrate that a stationary state of the process of clustering of nebulae can be reached only in periods longer than  $10^{18}$  years. Indeed, the *speed* with which stationary clusters are formed has nothing whatever to do with Lemaitre's theory.\* The problem of the speed of clustering is entirely of a *statistical nature* and the results obtained depend solely on the number of effective encounters among nebulae—that is, on those encounters during which appreciable amounts of the linear momenta of the involved nebulae are exchanged. Since the number and the effectiveness of such encounters depend only on the number of nebulae per unit volume, on the average velocity of these nebulae and on the law of interaction between nebulae (for which I assumed Newton's law of attraction), Lemaitre's considerations, which are also based on Newton's law of interaction between nebulae, cannot contribute anything to shorten the period necessary to achieve *statistically stationary* conditions in a large cluster of nebulae. As long as the law of interaction among nebulae is assumed to be Newton's law or any law of force not radically different from this law, a large cluster of nebulae can reach a stationary state only in a period far in excess of the time available in an expanding universe. The conclusion, therefore, remains intact, that the existence of statistically stationary configurations among clusters of nebulae is in contradiction with the hypothesis of an expanding universe.

<sup>1</sup> F. Zwicky, these PROCEEDINGS, 25, 604 (1939).

<sup>2</sup> M. S. Vallarta, these PROCEEDINGS, 26, 116 (1940).

<sup>3</sup> G. Lemaitre, these PROCEEDINGS, 20, 12 (1934).

\* Lemaitre's theory as far as I can see merely attempts to delineate the size and the material content of clusters of nebulae which may be formed in an expanding universe. It is not at all concerned with the question how long it takes for a large cluster to reach statistically stationary conditions.

## THE THEORY OF THE VISUAL THRESHOLD. II. ON THE KINETICS OF ADAPTATION

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I. The production of a neural effect resulting in visual discrimination on the basis of brilliance requires the summation of a very large number of elements of effect produced by a large number of excitable neural units. The expression of the cumulative resultant is in terms of "elements of action" which are definable by the analytical relation of excitability to time, intensity and other experimentally controlled variables. Sufficient objective evidence already exists to support the proposition that the intensity threshold of any one excitable unit intrinsically fluctuates. So also does its contributive efficiency in the production of the elements of effect. Excitability is defined as the reciprocal of the exciting intensity; it exhibits properties justifying the conclusion that, as so defined, it is proportional to a simple velocity constant.<sup>1</sup> From these considerations it is deduced<sup>2</sup> that the relation of photic excitability ( $1/\Delta I_0$ ) to exposure-time must appear as a probability integral in  $\log t_{exp.}$ . This is thoroughly in accord with the experimental data.<sup>3</sup>

II. By homologous reasoning it is deduced that threshold excitability ( $1/\Delta I_0$ ) should appear as a probability integral in  $\log t_D$ , where  $t_D$  is elapsed time during dark adaptation. The argument, condensed, is that at any instant the units potentially excitable form a frequency distribution of  $d(k)$ , where  $k$  is a velocity constant governing excitability; over the finite interval required for a measurement of excitability the production of elements of effect by units in a given  $d(k)$  class will decline with  $t_{exp.}$ , and the frequency distribution of elemental effects will then be one of  $-t_{exp.} d(k)$ , or  $-t_{exp.} d(1/t_0)$ , since  $k$  is proportional to a reciprocal time on the organism's time scale ( $t_0$ ); hence, a frequency distribution of  $a d \log t$ . Reasons have been given<sup>2</sup> for expecting that this distribution must be Gaussian. If  $t_{exp.}$  is constant, the total effect obtainable is a probability integral in  $\log I$ .<sup>4</sup> During recovery from light adaptation the frequency distribution of the excitabilities is conceived to form at any moment a frequency distribution of  $d(K)$ , where  $K$  is a momentary recovery velocity constant and proportional to  $1/t_0$ ; with passage of dark-time, the number of elements in a given  $d(K)$  class will decline, forming as a function of dark-time a frequency distribution in terms of  $-t_D d(1/t)$ , and thus of  $d \log t_D$ . At any time  $t_D$  during dark adaptation, the excitability, measured by  $1/\Delta I_0$ , must then also be measured by the integral of  $d \log t_D$  up to  $\log t_D$ . We may take this, likewise, to be Gaussian. The tests made are of two kinds: the ability of

this function to describe the data, and (more significant) the rational behavior of its three parameters when definite experimental conditions are modified.

The examination of this situation supplies a proof of the existence of random fluctuations in the individual excitabilities of elements of neural effect. Under various conditions of intensity and exposure time for light adaptation, the dark adapting elements of effect defined by  $d(1/\Delta I)/d \log t_D$

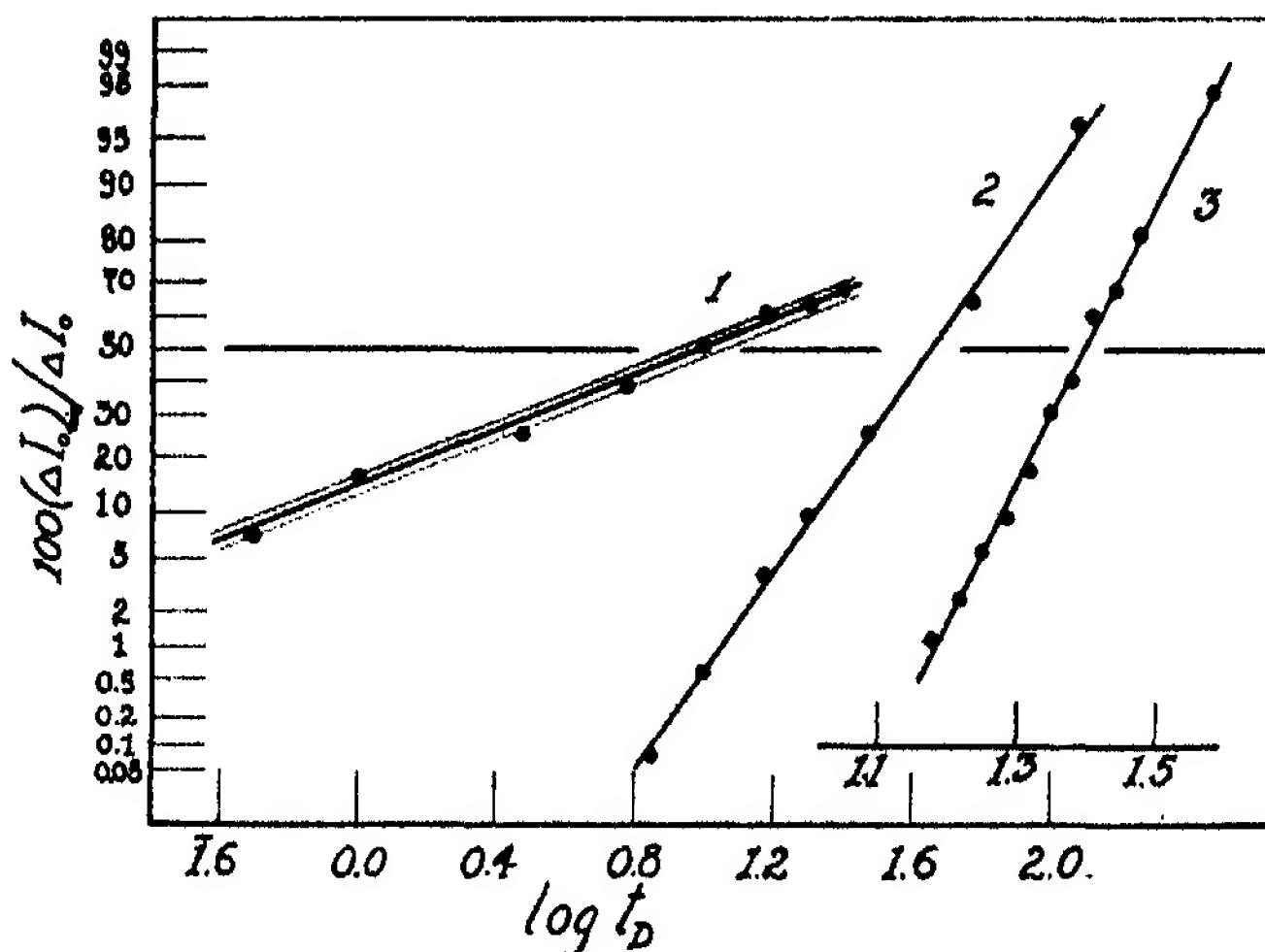


FIGURE 1

Dark adaptation contours from several sources, put on a probability grid. The abscissa is  $\log$  (dark-time, minutes); the ordinate is  $1/\Delta I_0$  as *per cent* of the maximum;  $\Delta I_0$  is the threshold intensity.

1—Foveal dark adaptation (red light); Kohlrausch.<sup>7</sup>

2—"Rod" dark adaptation; Dieter.<sup>8</sup>

3—"Rod" dark adaptation; Hecht, Haig and Chase<sup>9</sup> ("C. H.") violet light; after light adaptation to 400,000 *ml*.

The "cone" data are of course relatively more magnified; for 1,  $(\Delta I_0)_{\min.}$  is taken as 4615  $\mu L$ ; for 2,  $(\Delta I_0)_{\min.} = 5.755$  lux; for 3,  $\log (\Delta I_0)_{\min.} = 2.76$ .

do in fact form a complete symmetrical frequency distribution as a function of  $\log t_D$ . Moreover, the examination<sup>4</sup> of measurements of the retinal electrical response  $R$  shows that  $R$  is a probability integral in  $\log I$  for different dark-times. This is impossible unless the individual thresholds fluctuate at random.

It has been shown that when area of test-spot is enlarged<sup>5</sup> the S. D. of the function relating threshold intensity to exposure time *increases* for the "cone" elements but *decreases* for the "rod" elements. Similar contrasting

changes occur with the S. D. of the dark adaptation function.<sup>3</sup> This supplies a means of showing that dark adaptation is not a matter of increasing numbers of neural units available, but depends on the increasing potential production of elements of effect.

III. The visual threshold  $\Delta I_0$  follows the probability integral law as a function of dark-time in all available series of measurements with man and all other animals that have been tested. For these measurements, in general, no quantitative formulation has been available hitherto. The non-

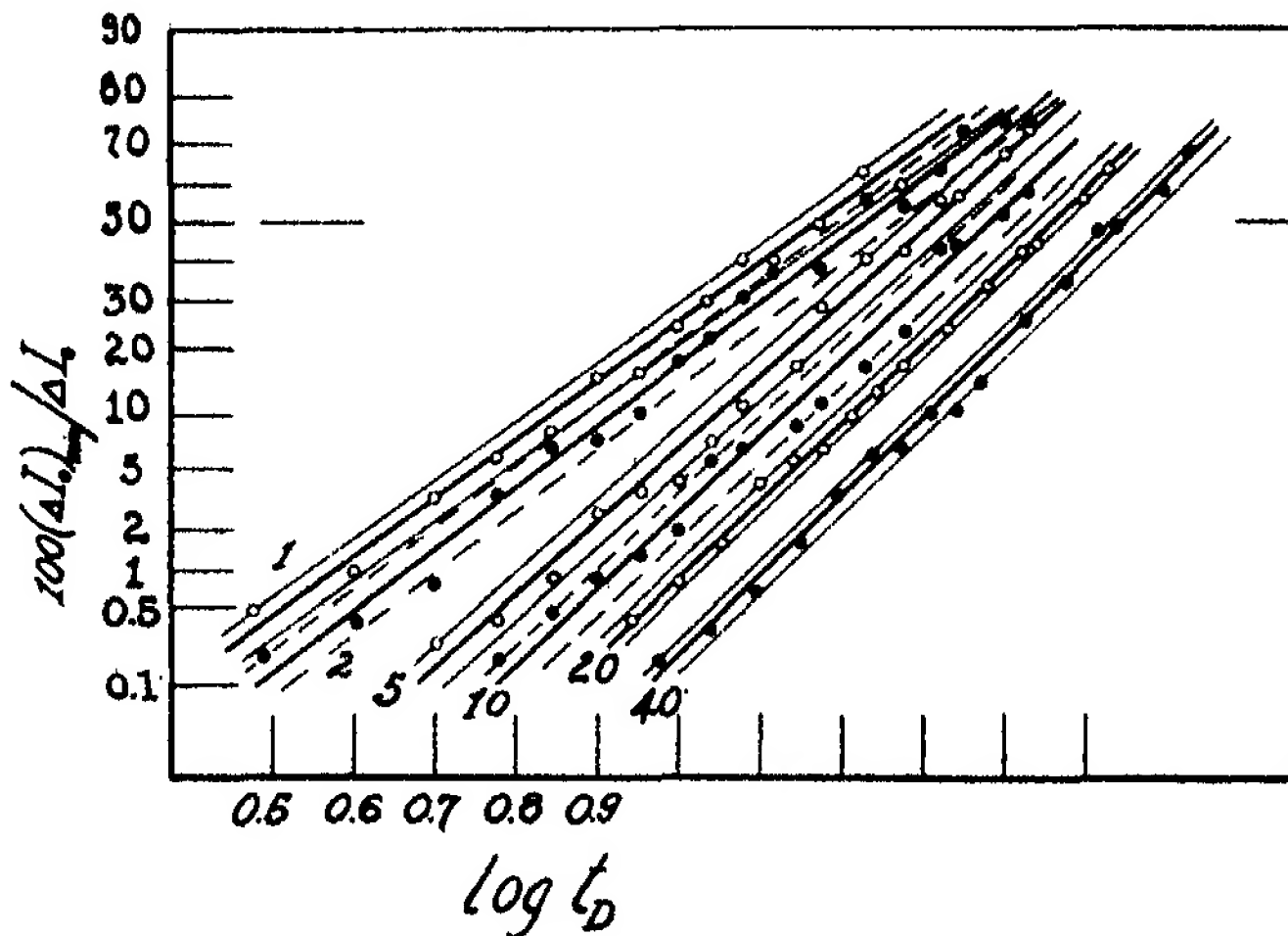


FIGURE 2

Data from Müller,<sup>10</sup> for dark adaptation of "rod" elements after light adaptation for 1, 2, 5, 10, 20 and 40 min. to 300 lux. The minimum value of  $\Delta I_0$  increases systematically,  $\log (\Delta I_0)_{\min.}$  being (in the same order): 0.18, 0.20, 0.20, 0.24, 0.28, 0.32. The value of  $\sigma'_{\log t_D}$  decreases in this order. Note also with increase of light-time the decrease in the width of the band measuring the statistically constant value of the relative scatter of  $t_D$  for each light-time; cf. <sup>3</sup>.

(The lines for  $t_L = 20$  and 40 min. have been moved, respectively, 0.1 and 0.2 log units to the right for clearness.)

specific form of the rule indicates that a general fact of biological organization determines its exhibition. This is found in cellular organization and in the known spontaneous but lawful fluctuation of cellular performance.

Several examples are given in figure 1. The three parameters of the probability summation are modified by the conditions of observation, but this does not alter the analytical form of the descriptive function.

The changing forms of the dark adaptation contours under various experimental conditions are also adequately described by this equation.

Systematic modifications of one or another parameter, or of several, are found as retinal region, test-spot area, antecedent adaptation, temperature, oxygen pressure and other variables are controlled. It is illustrated in this way<sup>5</sup> why these 3 parameters are necessary and apparently sufficient for the description of the dark adaptation contours. At the same time, there is provided a means of precisely and conveniently characterizing the nature of the influence exerted by each of these variables.

Several instances are given in figures 2, 3 and 4. For simplicity, the data illustrated in figures 2 and 3 refer only to the "rod" portions of the dark

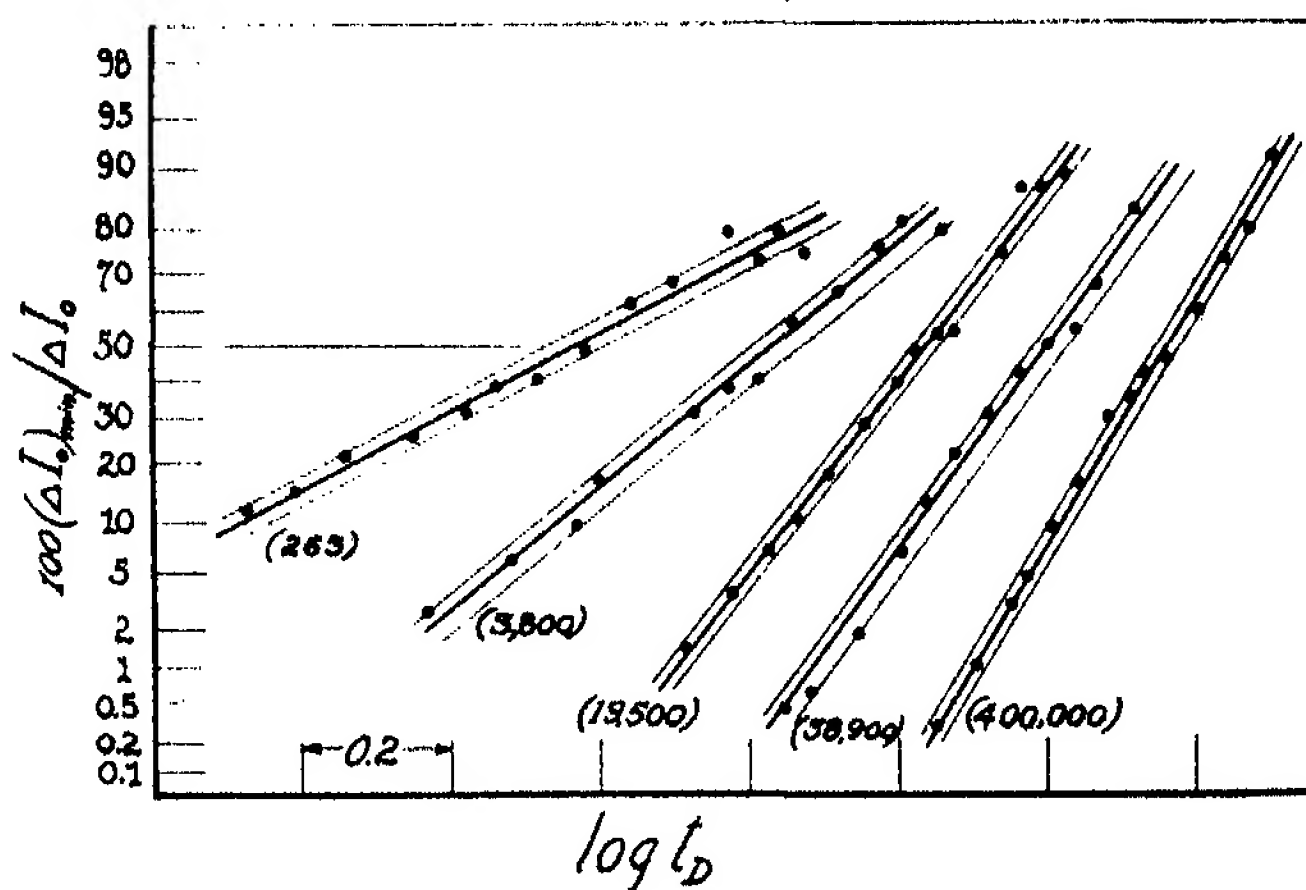


FIGURE 3

"Rod" dark adaptation after light adaptation to different intensities (photons) for a fixed time (2 min.), from Hecht, Haig and Chase;<sup>9</sup> averaged monocular determinations of  $\Delta I_0$  with violet light (data on S. H.). The values for  $\log (\Delta I_0)_{\min.}$ ; from left to right, are: 2.40; 2.37; 2.53; 2.78; 2.74. The slope (i.e.,  $1/\sigma'$ ) decreases with increasing light adaptation, the abscissa of inflection increases and the horizontal breadth of the band decreases. The plots are shifted laterally for clearness.

adaptation contours. When the time of exposure to an adapting light is increased, or the intensity of the adapting light, the homogeneity of the frequency distribution of effect thresholds in terms of  $\log t_D$  is increased, although its total size (maximum  $1/\Delta I_0$ ) is less. Under reduced oxygen pressure<sup>11</sup> the population also becomes more uniform but is of decreased total size. This is directly accounted for by the elimination of the most light-sensitive, and consequently the most slowly adapting, elements as a result of the lowering of oxygen pressure.

The action of reduction in vitamin-A intake is of a slightly different character (Fig. 4). Here, the total number of available units is decreased

and its standard deviation ("rods") is decreased or ("cones") increased, but the abscissa of its inflection point changes comparatively little. The detailed examination of these matters is considered in another place.<sup>3</sup>

IV. The photochemical hypothesis of the visual threshold<sup>7</sup> postulates that its quantitative properties are determined by the receptive mechanism

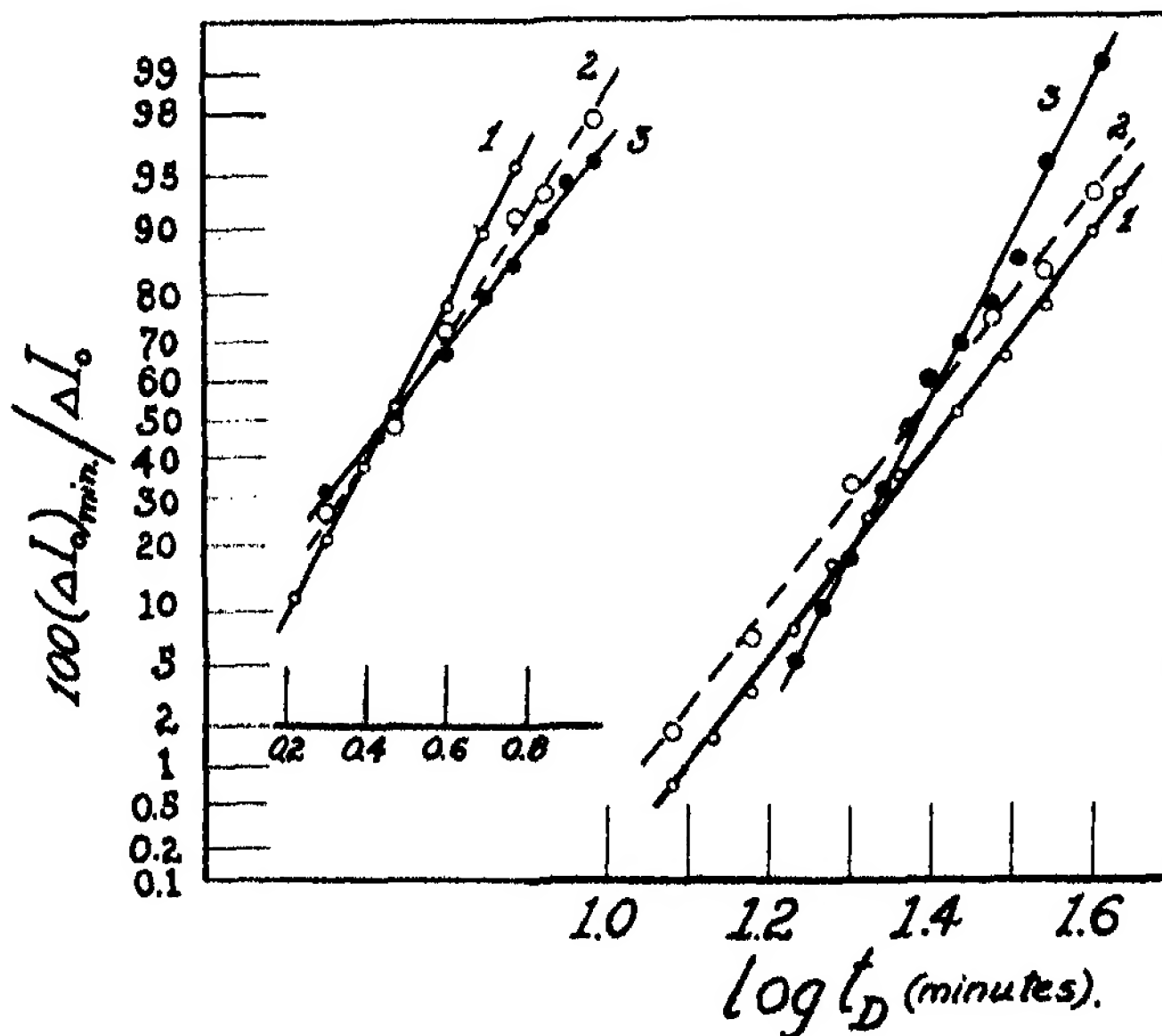


FIGURE 4

The effect on "cone" (to the right) and "rod" (to the left) dark adaptation produced by experimental restriction of dietary vitamin-A. These data have been published graphically by Wald, Jeghers and Arminio.<sup>12</sup> The lines 1 refer to the initial normal state, the points being read from a graphical analysis<sup>8</sup> of a number of sets of the measurements with the left eye of one subject. The lines 2 carry single observations after 15 days' restriction of vitamin-A; lines 3, after 22 days. Note that the increase of slope (i.e., decrease of  $\sigma'_{\log t_D}$ ) found in the "rod" data is reversed for the "cones;" this is characteristic of the "integration effect" for these 2 populations of elements,<sup>3</sup> and is the typical result of increasing their number. Here, however, it is accompanied by a decrease of total number, and points merely to a facilitation of the integration effect accompanying incipient neural injury.

at the retina, and that these properties describe the reaction-velocity kinetics of a physicochemically homogeneous system involving a photolabile substance in equilibrium with its decomposition products. The use of this hypothesis has involved further suppositions, for example that the reciprocal of the exciting intensity, or of its logarithm, is a measure of the



sensory effect produced; and basically and explicitly that the occurrence of threshold effect ("absolute" or differential) corresponds to a fixed, "constant" amount of sensory and photochemical effect. No reason is found for the acceptance of any of these assumptions, and the assumption of threshold effect constancy is contradicted.<sup>2</sup> The statistical theory makes none of these assumptions, provides an accurate description of the data of dark adaptation, and interprets quantitatively the changes induced by experimental treatments. This the photochemical hypothesis does not do.

The statistical theory also accounts automatically for the facts that the same analytical form of the dark adaptation contour obtains with all animals tested, and that the same kinds of changes are produced—for example, by the conditions of light adaptation—in insects and in man.

V. *Summary.* The kinetics of dark adaptation, as expressed in the change of intensity threshold with dark time, displays the statistical result of fluctuating recovery of excitability as regards the elements of neural effect produced from excited units with variable thresholds. The form of the dark adaptation contour does not reveal the physiochemical nature of the metabolic process governing receptor excitation. It is simply a Gaussian probability integral in which  $1/\Delta I_0$  is the ordinate and  $\log(\text{dark-time})$  is abscissa. This can be arrived at deductively. The behavior of its three parameters (maximum  $[1/\Delta I_0]$ ; abscissa of inflection; standard deviation of the first derivative) under different experimental conditions of adaptation and of its testing, in various animals, is in accord with this.

<sup>1</sup> Crozier, W. J., *Proc. Nat. Acad. Sci.*, **25**, 78 (1939); Crozier, W. J., and Wolf, E., *Ibid.*, 171 (1939).

<sup>2</sup> Crozier, W. J., *Proc. Nat. Acad. Sci.*, **26**, 54 (1940).

<sup>3</sup> Crozier, W. J., "The Kinetics of Adaptation," to be published elsewhere (1940).

<sup>4</sup> E.g., data by Riggs, L. A., *Jour. Cell. Comp. Physiol.*, **9**, 491 (1937); analyzed in <sup>3</sup>.

<sup>5</sup> Crozier, W. J., "The Kinetics of Adaptation," to be published elsewhere (1940).

<sup>6</sup> Hecht, S., *Physiol. Rev.*, **17**, 239 (1937).

<sup>7</sup> Kohlrausch, A., *Pfl. Arch. ges. Physiol.*, **196**, 113 (1922).

<sup>8</sup> Dieter, W., *Pfl. Arch. ges. Physiol.*, **222**, 381 (1929).

<sup>9</sup> Hecht, S., Haig, C., and Chase, A. M., *Jour. Gen. Physiol.*, **20**, 831 (1936-1937).

<sup>10</sup> Müller, H. K., *Arch. Opth.* Berlin, **125**, 624 (1931).

<sup>11</sup> Measurements by: McFarland, R. A., and Evans, J. H., *Amer. Jour. Physiol.*, **127**, 37 (1939); cf. <sup>4</sup>.

<sup>12</sup> Wald, G., Jeghers, H., and Arminio, J., *Amer. Jour. Physiol.*, **123**, 732 (1938).

## EFFECTS OF CERTAIN CHEMICAL TREATMENTS ON THE MORPHOLOGY OF SALIVARY GLAND CHROMOSOMES AND THEIR INTERPRETATION

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The difficulties met with when trying to relate the morphological structure of salivary and other chromosomes to known physico-chemical facts regarding the constituent proteins and nucleic acid molecules have suggested an attack involving the observation of chemical reactions within the chromosomes.

We subjected the salivary gland chromosomes of *Drosophila melanogaster* to treatment with solutions of varying pH ranging from 14 to 1 (i.e., 1 *N* base to 1 *N* acid). The results of these experiments, in the light of what is known about the behavior of proteins and nucleic acids toward acid and alkali, have enabled us to make some suggestions regarding the morphological as well as chemical structure of the salivary gland chromosome.

We shall present first the experiments (Kodani and Calvin), then the morphological interpretation (Goldschmidt) and finally the chemical interpretation (Calvin).

1. *Experimental.*—A series of buffered NaOH solutions were made up between pH 9.2 and pH 14. The solutions of pH 9.2, 9.6, 10.0 and 10.4 were borate buffers; those of pH 11.0, 11.4 and 12.0 were phosphate buffers; those of pH 12.4, 12.7 and 12.9 were glycine-NaCl buffers prepared according to Sørensen's method;<sup>1</sup> and those of pH above 13.0 made by diluting a normal solution of NaOH. The salivary glands were dissected from larvae in Ringer solution and then transferred to a drop of solution of given pH on a slide and treated for various lengths of time. The glands were then stained with aceto-carmines for several minutes and smeared on the slide. The time of treatment varied from 1 to 150 minutes. About 700 glands were studied.

The first observable change in structure of chromosomes after treatment is a longitudinal condensation and slight swelling which occur simultaneously so that the chromatic bands come closer to each other and break up into discrete dots (Fig. 2, *A* and *A1*). Thus the chromosomes lose their characteristic striations entirely, although they still retain a more or less clear outline. The time required to produce this structure is different for different pH's. The relative times and concentrations necessary to initiate this change are shown in figure 1. It should be noted that various points on the curve indicate merely the average times for corresponding pH's.

The area above the curve represents the region of chromosome change.

Within this area it may be noticed that the chromosomes undergo a definite sequence of changes as the time of treatment is increased. It is also found that the type of sequence is different in three different ranges of pH, namely, above 13.1, between 13.0 and 12.6 and below 12.6. The points of division into these pH ranges are not critical but represent simply the range in which there appears a definite preponderance of one type of sequence over the others. Each sequence is essentially the same for all pH's within each range, and each sequence can be divided into stages.

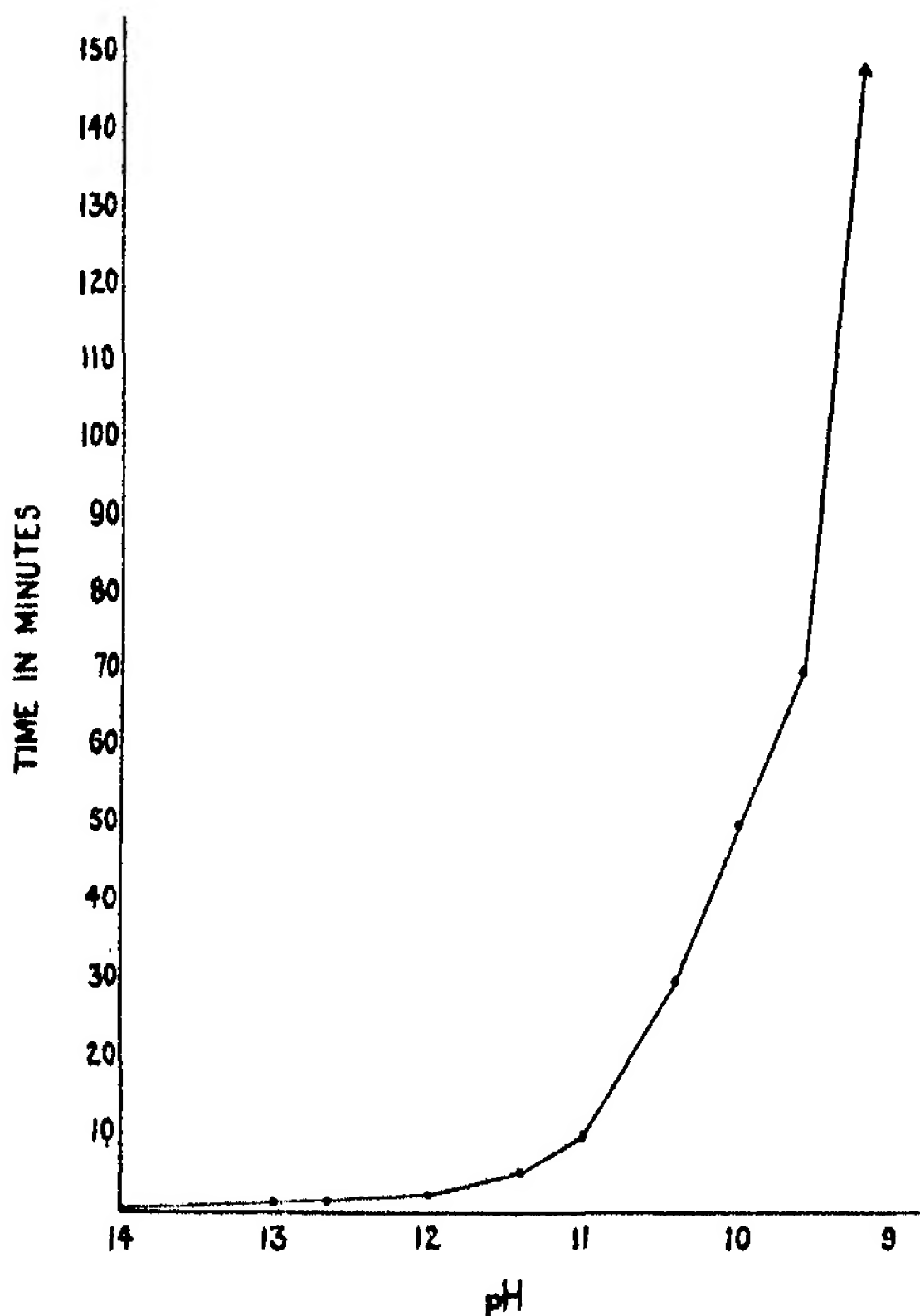


FIGURE 1

The curve indicates the relation between time and pH required to produce initial change.

The initial change described above is common to all three ranges of pH. Within the highest range of pH (above 13) this is succeeded by the following three stages. The first of these may be called the lampbrush stage because of its striking similarity to the situation in lampbrush chromosomes. At the beginning of this stage the chromosome typically shows two longitudinal

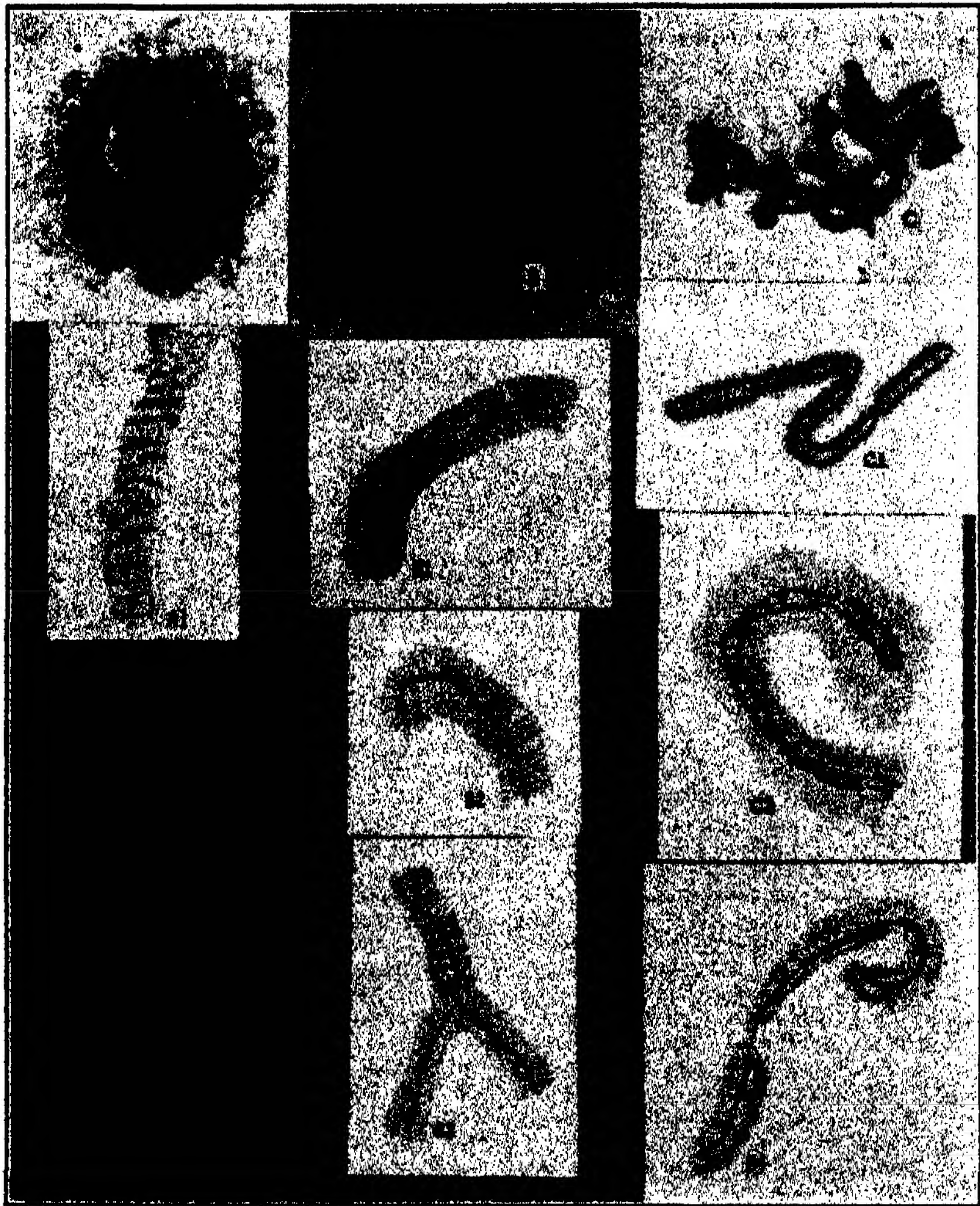


FIGURE 2

*A*, photomicrograph of chromosomes in Stage 1. *A1*, camera-lucida drawing of a part of chromosome in the same stage. *B*, photomicrograph of typical lampbrush chromosomes. *B1*, camera-lucida drawing of the chromosome structure at very early lampbrush stage. *B2*, camera-lucida drawing of the structure subsequent to *B1*. *B3*, camera-lucida drawing of a part of a chromosome showing differential contraction. *B4*, camera-lucida drawing of lampbrush structure from photomicrograph *B*. *C*, photomicrograph of the ladder stage. *C1*, camera-lucida drawing to show details of the early ladder stage. *C2*, camera-lucida drawing of a part of a chromosome in the late ladder stage.  $\times$  ca. 1350 for all camera-lucida drawings and  $\times$  ca. 1000 for all photomicrographs.



beaded strings (chromatids), paired throughout their length, with each pair of homologous chromomeres connected by a thin, sometimes beaded, transverse thread, which extends on either side to the margin of the chromosome (Fig. 2, *B1*). Subsequently the chromatids synapse, with the lateral threads still remaining in the same position (Fig. 2, *B2*). Although these steps may be seen in the same preparation, the proper sequence is indicated by relative frequencies of occurrence with respect to time and by the synapsed chromatids of the succeeding steps. The chromosome now begins to contract longitudinally. This process is suggested by the appearance in different parts of the chromatid of groups of larger chromomeres, from each of which there extend a number of lateral threads (Fig. 2, *B3*). Each of these larger chromomeres is composed of a number of smaller chromomeres, as indicated by the direct correlation between size of chromomeres and number of lateral threads. The longitudinal contraction of the chromatid proceeds irregularly at first but finally assumes the uniformly shortened appearance indicated in figure 2, *B4* and *B*. This typical lampbrush structure marks the end of the stage. It should be noted that although only four strands are shown in figure 2, *B3*, the presence of a quadripartite structure may be seen very early in the lampbrush stage and may be considered to be present throughout.

In the next stage the chromosomes lose their lampbrush structure and finally become invisible. Occasionally, however, the chromosomes retain the outline in faint red color after the chromatic strings disappear, but after prolonged treatment these also dissolve without further change in structure.

Within the middle range of pH (13.0-12.7) the following three stages are observed. (1) Same as stage 1 in the previous range of pH. (2) At the beginning of this stage the chromosome is characterized by two thick strings which are connected by a number of fine bridges along their length, with numerous short hairs, which may be absent in some preparations, extending from the strings to the outer side. This structure of the chromonemata is retained until the next stage sets in, except that toward the end of this stage the hairs often become very much extended (Fig. 2, *C* and *C2*). This stage may be called the ladder stage because of its ladderlike structure. (3) The chromosome loses the ladderlike structure and finally becomes indistinct.

Within the lowest range of pH (below 12.6), the chromosomes go through the following two stages only. (1) The same as stage 1 of the previous ranges of pH. (2) The chromosomes disappear in the nucleus without further change in structure. In some chromosomes, however, it is occasionally observed that the achromatic regions dissolve without previous condensation or swelling, so that the chromosomes fall apart into bands and granules.

The time required to initiate various stages varies with pH, and for all three ranges the higher the pH the earlier the stages begin. Within the highest range of pH the lampbrush stage begins within five minutes for all pH's and ends within about two hours and a half. In the middle range the ladder stage starts ten to thirty minutes after the beginning of the treatment and seems to persist about the same length of time as the lampbrush stage. The times stated here were determined at room temperature. Change of temperature considerably alters the occurrence and persistence of stages. A detailed investigation of this problem is now being made.

The presence of nucleic acid in the regions which take acetocarmine stain in treated chromosomes was confirmed by staining with Feulgen-Rossenbeck reagent. The existence of protein in treated chromosomes is shown by the fact that they are stained with ninhydrin. The exact distribution of the proteins at various stages of the treated chromosomes is now being studied. A number of preparations stained with Sudan IV indicated the presence of small amounts of lipoid in the native chromosomes, which were removed immediately on contact with alkali.

There seems to be no specific effect of the various ions used in the several buffers. Treatment with 1 *N* NaCl solution produced no changes in structure comparable to those of the alkali. There is, however, a general shrinkage of the chromosome after prolonged treatment with this concentrated salt solution, without any definite change in the relative orientation of the various parts. This shrinkage seems to be due simply to the dehydration resulting from the hypertonic character of the salt solution. On the other hand, normal HCl produced no visible changes even after prolonged treatment. Alkalis, such as KOH and NH<sub>4</sub>OH, were found to produce the same effect as that of NaOH, indicating that it is the OH ion that produces the effect.

2. *Morphological Interpretation.*—How can these facts be interpreted morphologically in connection with known facts? Two major possibilities become visible: either the lampbrush structure is not preformed in the salivary chromosome but is an artefact produced by the treatment, or the structure is present in the salivary chromosome and made visible by the treatment. A comparison with the typical lampbrush chromosomes of the ovocytes of lower vertebrates (Rückert,<sup>2</sup> Carnoy-Lebrun,<sup>3</sup> Maréchal,<sup>4</sup> Koltzoff<sup>5</sup>) strongly suggests the second assumption. It is, moreover, made practically a certainty by Painter's<sup>6</sup> discovery, published while this paper was being prepared for the press, that in nurse-cell nuclei of Diptera a lampbrush structure of the chromosomes is present normally. As all the figures of the lampbrush chromosomes indicate, they consist of a central single or double chromonema with chromomeres, which, in ovocytes, is undoubtedly the stretched prophase chromosome, which later contracts into the metaphase chromosome of the meiotic division. The bristles of



the lampbrush may appear as loops attached to the chromomeres and, if covered with chromatin, may even look like transverse discs (see especially

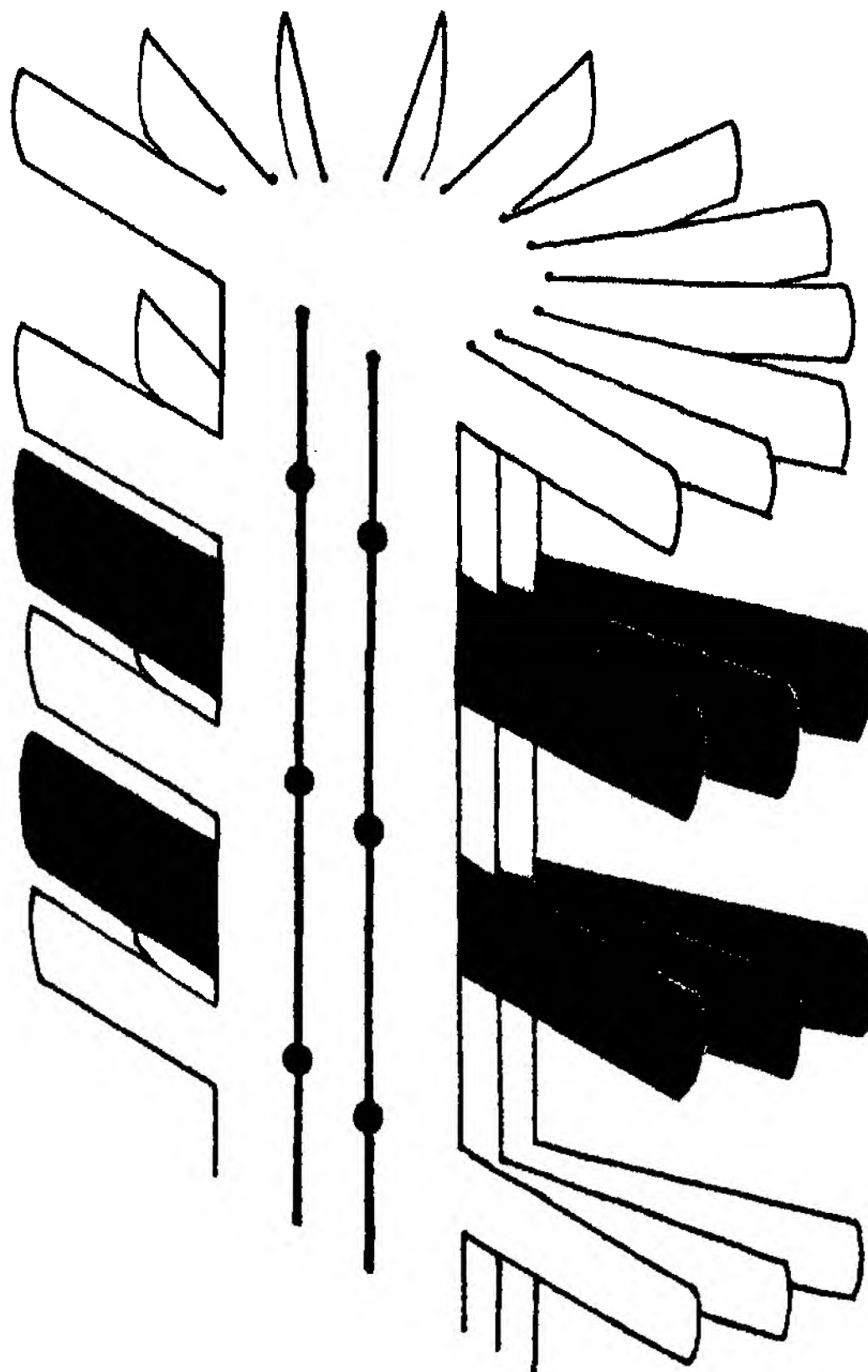


FIGURE 3

Diagrammatic representation of the arrangement of chromonemata and nuclein in the salivary chromosomes. With minor changes the diagram applies also to lampbrush chromosomes of ovocytes. One-half chromosome represented; the two main chromatids in thick lines; part of the bundle of other chromonemata in thin lines; nuclein, dark without referring to detailed structure. Two possibilities discussed in text are shown on right and left side of the diagram.

Carnoy-Lebrun and Koltzoff). The interpretation of Rückert, later elaborated by Goldschmidt,<sup>7</sup> was that in this condition trophochromatin is separated from idiochromatin and sloughed off into the nucleus, to be used

as material for the growth of the egg. This old interpretation was elaborated in a more modern way by Koltzoff, who considers the sloughed-off loops as replicas of the chromonema. The recent knowledge regarding intranuclear multiplication of the chromatids by division without nuclear division as visible in salivary and other gland nuclei (Geitler, a.o.<sup>6</sup>) suggested a similar interpretation for the lampbrush chromosomes, namely, a multiple division of the stretched chromatid into a bundle of chromonemata of which always one (or two) remain intact as the future meiotic chromosomes (idiochromatin) and retain their not completely stretched condition, whereas all others stretch completely, therefore form loops and are sloughed off as trophochromatin. This slight modification of Koltzoff's description was used in the lectures of one of the authors. The recent paper by Painter contains the part of this interpretation which relates the lampbrush to multiple division of the chromonemata, which he assumes to lead to a storage of nucleic material in the cytoplasm to be used during development for the synthesis of chromosome material. (Painter points here to the physiologically identical but morphologically different behavior in those cases in which eggs grow by assimilation of nurse cells. Much important material pointing in the same direction can be found in Goldschmidt's old papers on trophochromatin and idiochromatin,<sup>7</sup> as well as viewpoints which deserve reinvestigation, starting with our present knowledge.)

Confronting, now, the facts regarding the morphology of the lampbrush chromosomes with both the experimental and morphological facts reported in the first part of this paper, as well as with Painter's recent discoveries, we come to a picture of the morphology of the salivary chromosomes as represented in the diagram (Fig. 3). The double chromonema (four in the combined salivary chromosomes), which represents the actual chromatid, is present but not visible in the untreated salivary chromosome on account of the distribution of the chromatic material (the discs). It becomes visible only when the action of the alkali concentrates the nucleic acid upon these primary chromonemata (Fig. 2). The other chromonemata form the loops (the bristles of the lampbrush), being stretched to full length and therefore coiling into loops between the serial points of attraction to the main chromonema, presumably its chromomeres. These bristles or loops are invisible in the normal condition in the salivaries (though visible in the nurse cells, according to Painter) because of their incrustation with nuclein. (Again, as in the lampbrush chromosomes of the oocytes, the main chromonemata represent the idiochromatic part of the chromosome and the unfolded lampbrush chromonemata the trophochromatic part, functioning in the special function of the gland cell.)

One point, however, cannot be decided yet, i.e., the details of the arrangement of the nuclein (nucleic acid) in the form of dark bands. The

facts, as represented in the figures, suggest that the loops are attached to the chromomeres of the central chromonema and therefore represent the core of the chromatic discs. But it is possible that this picture is a result of the contraction after treatment and that in the natural condition the chromatic discs would be found between the loops, as indicated as an alternative possibility on the left side of the diagram. This latter assumption could be brought in line more easily with some morphological observations (Bauer<sup>9</sup>) as well as with the polariscopic facts (Schmidt<sup>10</sup>). Further experiments might furnish the decision which is hardly to be reached by pure observation. It might finally be pointed out for future use that the individual loops of the lampbrush might represent individual polypeptid chains (see also Koltzoff). In this case their order of magnitude would be 10–30,000 Å and the whole chromonema would be a superchain of ca. 1000 such chains.

3. *Chemical Interpretation.*—Keeping in mind the spectroscopic and enzymatic experiments of Caspersson<sup>11</sup> and his co-workers, the optical experiments of Schmidt,<sup>10</sup> the stretching experiments of Buch<sup>12</sup> and the enzymatic experiments of Mazia and Jaeger,<sup>13</sup> we can extend somewhat the suggestions based on the x-ray work of Astbury and Bell<sup>14</sup> regarding the molecular organization of the chromonema in the light of the present results. Other models, e.g., Wrinch,<sup>15</sup> based upon other suggestions of protein structure, have been discussed by Gulick<sup>16</sup> and are rejected, since they cannot be brought into accordance with all the various facts just noted.

The essential facts derived from the present experiments are the following: (a) the sensitivity of the chromosome to alkali, together with its comparative stability toward salt and acid; (b) the sharp decrease in the rate of disintegration as the pH falls below 10; (c) the initial breaking up of the transverse nucleic acid bands into discrete spots; (d) the subsequent condensation of the nucleic acid into longitudinal strings (four strings in each chromosome pair; (e) the appearance of a lampbrush-like structure of the protein around the longitudinal strings; (f) the occasional appearance at certain pH's of a comparatively undisturbed chromosome skeleton containing no nucleic acid. (Compare with Mazia and Jaeger.)

Following Astbury and Bell, the most important elements in the molecular organization of protein fibres are the partially folded and partially extended polypeptid chain, together with the fitting of the nucleic acid onto the extended portion of the chain. These chains are held together by at least two different kinds of bonds. On the one hand there must be large groups of chains held together in a bundle by essentially primary valence bonds through the various amino acid side groups of the polypeptide chain; and on the other hand there must also operate between these primarily bound groups of chains, secondary weaker bonds which are very susceptible

to chemical or other agents. These secondary bonds are the ones which are released by alkali, and the fact that the pH at which this effect begins to occur corresponds quite well with the isoelectric point of nucleoprotein seems to indicate that we are here dealing with a neutralization of the guanidine ion of the arginine residue which is so prevalent in nucleoproteins. The fact that this initial reaction of the chromosome to alkali has practically no temperature coefficient (a result of a part of our experiments not included in this paper), whereas the following reactions do, is confirmation of the suggestion that we are indeed concerned with a neutralization reaction. This also may account for the fixing property of acids. It is interesting to note in this connection the suggestion of Drawert<sup>17</sup> that the chromosome is actually in a very acid condition, the pH being between 3 and 5, so that, in alkaline solutions the hydrogen atom forming the bond would be neutralized and so reduce the attraction between groups of chains, and thus destroy the forces mainly responsible for the gross visible structure of the chromosome. The conditions of the experiment are much too mild to attack a peptide link or an ester. The initial contraction along the length and swelling of the chromosome, together with the breaking up of the chromatic bands (nucleic acid), is thus understood in terms of the relaxation of the forces maintaining the polypeptide chains in their relationship to one another and to the nucleic acid.

It should be kept in mind that none of the various structures herein described resulting from the treatment with alkali represent a condition of equilibrium of the chromosome or its constituents at the particular pH of the solution in which it was immersed. The contact with alkali doubtless started a number of parallel or consecutive chemical reactions which the fixation in acetic acid stopped at some particular point. The resulting observable condition is thus the product of several reactions whose rates most likely have quite different dependencies upon pH as well as temperature, so that it is not necessary that the identical sequence of changes be observable at different pH's differing only in the rate of their appearance. The unraveling of the various possible reactions, for example, the hydrolysis of the esters as well as the breaking of hydrogen bonds or salt links, awaits further experiment.

The next major stage in the sequence of changes is the condensation of the nucleic acid into dense longitudinal strings. This tendency of flat aromatic molecules to associate with each other, plane upon plane into long threads, is characteristic and has been observed for aqueous solutions of sodium thymonucleate as well as larger flat molecules.<sup>18, 19</sup> This takes place after the restraining forces have been released and the nucleic acid is free to move, either remaining attached to a loosened polypeptide chain, or entirely free of it. The fact that this condensation of nucleic acid always results in two longitudinal strings for each chromosome pair

may mean, as has been suggested in the morphological discussion, that these two strings have a primary independent existence in the untreated chromosome, or that there are simply two types of polypeptide chains together with their corresponding acids, and that there is no mixing of the two in the condensation process.

It is hoped that these and many other questions will receive an answer as further chemical experiments of this type are performed.

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# ABELIAN GROUPS WHICH CONTAIN NO MORE THAN 25 PROPER SUBGROUPS

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If a group  $G$  is not the identity it contains at least two subgroups, viz., the identity and the entire group. These two subgroups are said to be improper subgroups of  $G$  while each of its other subgroups, when the order of  $G$  exceeds 2, is called a proper subgroup of  $G$ . The identity is known as an *actual* or *real* subgroup of  $G$  so that  $G$  has always one more actual subgroup than its proper subgroups, whenever  $G$  is not the identity. When  $G$  is abelian and has an order which is not a power of a prime number it is the direct product of its Sylow subgroups and the number of its subgroups is the product of the numbers of the subgroups of its Sylow subgroups. If  $G$  is the cyclic group of order  $p^m$ ,  $p$  being an arbitrary prime number, then it contains exactly  $m - 1$  proper subgroups. The following elementary theorem is frequently useful in the determination of the number of the proper subgroups of a given group. *A necessary and sufficient condition that there exists at least one cyclic group whose order is not a power of a prime number which contains  $k$  but no more than  $k$  proper subgroups, where  $k$  is an arbitrary positive integer, is that  $k + 2$  is not a prime number.*

From the preceding paragraph it results that in order to determine the number of the abelian groups which separately involve a given number  $k$  of proper subgroups we may first find the prime power abelian groups which contain exactly  $k$  proper subgroups, then find the different possible sets of positive integral factors of  $k + 2$  and determine the number of prime power abelian groups for each of these sets of factors. Hence we shall first determine the numbers of the subgroups of prime power abelian groups which separately involve no more than 25 proper subgroups. The simplest case is the one in which the abelian group is of order  $p^m$  and of the type  $1^m$  since in this case there is a well-known formula for the number of the subgroups. When  $m > 3$  the number of the distinct subgroups exceeds 25 for every value of the prime number  $p$ . This is also true when  $m = 3$  except when  $p = 2$ . In this special case it is known that  $G$  contains 14 proper subgroups. When  $m = 2$  there are  $p + 1$  proper subgroups in  $G$  and for our purpose it may be assumed that  $p < 29$ . The number of the subgroups as  $p$  varies from 2 to 23 is as follows: 3, 4, 6, 8, 12, 14, 18, 20, 24.

When  $G$  is of type  $k, 1$  the number of the proper subgroups of  $G$  is  $kp + k$ , where it may be assumed that  $1 < k < 9$ . As  $k$  varies from 2 to 8, and  $p$  is restricted to values so as to make the total number of proper subgroups less than 26, the number of proper subgroups is as follows: 6, 8, 12, 16, 24;



9, 12, 18, 24; 12, 16, 24; 15, 20; 18, 24; 21; 24. When  $G$  is of type  $k$ , 2 the value of  $k$  is either 2 or 3 since otherwise there would be more than 25 proper subgroups. When  $k = 3$  then  $p = 2$  and there are 20 proper subgroups in  $G$ . When  $k = 2$  there are  $p + 1$  subgroups of order  $p$ ,  $p^2 + p + 1$  of order  $p^2$ , and  $p + 1$  of order  $p^3$ , making a total of  $p^2 + 3p + 3$  subgroups. This exceeds 25 whenever  $p > 3$ , and is 13 or 21 as  $p = 2$  or 3, respectively. The only case that remains to be considered is when  $p = 2$  and  $G$  is of type 2, 1, 1. In this case there are 25 proper subgroups in  $G$  since there are 7 of order 2, 11 of order 4 and 7 of order 8.

By means of these results and the theorem stated at the close of the first paragraph of this article it is easy to determine all the abelian groups which separately contain no more than 25 proper subgroups. When the number of these subgroups is less than 15 the corresponding abelian groups are included in the lists of all the possible groups which involve a given small number of proper subgroups, which were published by the present writer in recent numbers of these PROCEEDINGS, beginning with volume 25, page 367 (1939). If an abelian group contains exactly 15 proper subgroups it follows from the preceding paragraphs that it is either the abelian group of order 64 and of type 5, 1 or the cyclic group of order  $p^{16}$ ,  $p$  being any prime number. From the lists noted above it results that there are exactly three prime power abelian groups which separately contain exactly 16 proper subgroups. Besides the cyclic group of order  $p^{17}$ , where  $p$  is an arbitrary prime number, these are the abelian group of order  $7^8$  and of type 2, 1 and the abelian group of order  $3^6$  and of type 4, 1.

If an abelian group whose order is not a power of some prime number contains exactly 16 proper subgroups it contains 18 subgroups altogether. It may therefore be any one of the three cyclic groups whose separate orders are  $p_1 p_2^2 p_3$  ( $p_1$ ,  $p_2$  and  $p_3$  being distinct prime numbers),  $p_1 p_2^8$  or  $p_1^2 p_2^5$ . It may also be the direct product of the non-cyclic group of order 9 and the cyclic group of order  $p^2$ , where  $p$  is an arbitrary prime number with the exception of 3. Hence *there are seven abelian groups which separately contain exactly sixteen proper subgroups*. One of these is composed of a triply infinite system of individual groups, two of them are composed of doubly infinite systems, two of simply infinite systems, while two are individual groups. If an abelian group contains exactly 17 proper subgroups its order is a power of a prime number since  $17 + 2$  is a prime number. It is therefore the cyclic group of order  $p^{18}$ , where  $p$  is an arbitrary prime number.

It was noted above that there are four prime power abelian groups which separately contain exactly 18 proper subgroups. These are the cyclic group of order  $p^{19}$ , where  $p$  is an arbitrary prime number, the non-cyclic group of order  $17^2$ , the abelian group of order  $5^4$  and of type 3, 1, and the abelian group of order  $2^7$  and of type 6, 1. If an abelian group which contains exactly 18 proper subgroups has an order which is not a power of a

prime number it may be the direct product of the cyclic group of orders  $p_1$ ,  $p_2$  and  $p_3^4$ ,  $p_1$ ,  $p_2$  and  $p_3$  being distinct prime numbers. Whenever its order is divisible by three distinct prime numbers it may also be the direct product of the four group and the groups of orders  $p_1$  and  $p_2$ , respectively, where  $p_1$  and  $p_2$  are arbitrary distinct odd prime numbers. When its order is divisible by only two distinct prime numbers it may be either the cyclic group of order  $p_1^3 p_2^4$ , where  $p_1$  and  $p_2$  are arbitrary distinct prime numbers or the cyclic group of order  $p_1 p_2^9$ .

It may also be the direct product of the four group and the cyclic group of order  $p^3$ , where  $p$  is any odd prime number, or the direct product of the non-cyclic group of order 49 and the group of any prime order except 7, or the group of order 27 and of type 2, 1 and the group of any prime order except 3. Hence *there are eleven distinct abelian groups which separately involve exactly eighteen proper subgroups*. One of these is a triply infinite system of groups, three are doubly infinite systems, four are simply infinite systems, while the remaining three are individual groups. If an abelian group contains exactly 19 proper subgroups and its order is a power of a prime number it is the cyclic group of order  $p^{20}$ . If its order is not a power of a prime number it is the direct product of two Sylow groups which involve 3 and 7 subgroups, respectively. It is therefore the cyclic group of order  $p_1^2 p_2^6$ ,  $p_1$  and  $p_2$  being distinct prime numbers.

The 4 prime power abelian groups which separately contain 20 proper subgroups are the cyclic group of order  $p^{20}$ ,  $p$  being any prime number, the non-cyclic group of order  $19^2$ , the abelian group of order  $3^6$  and of type 5, 1, and the abelian group of order  $2^6$  and of type 3, 2. If an abelian group whose order is not a power of a prime number contains 20 proper subgroups it is either the cyclic group of order  $p_1 p_2^{10}$ ,  $p_1$  and  $p_2$  being distinct prime numbers, or the direct product of the group of any odd prime order and the abelian group of 16 and of type 3, 1. Hence *there are six abelian groups which separately contain exactly twenty proper subgroups*. If an abelian group contains exactly 21 proper subgroups it is a prime power group and hence it is one of the following three groups: the cyclic group of order  $p^{22}$ ,  $p$  being any prime number, the abelian group of order  $2^8$  and of type, 7, 1, or the abelian group of order  $3^4$  and of type 2, 2. If a prime power abelian group contains exactly 22 proper subgroups it is the cyclic group of order  $p^{23}$ ,  $p$  being any prime number. If the order of such a group is not a power of a prime number then it is the direct product of prime power groups involving one of the following sets of numbers of subgroups such that the subgroups in each set have orders which are powers of distinct prime numbers: 2, 2, 2, 3; 4, 2, 3; 8, 3; 2, 2, 6; 4, 6; 2, 12.

The fourfold infinite system of cyclic groups of order  $p_1 p_2 p_3 p_4^3$ ,  $p_1$ ,  $p_2$ ,  $p_3$ ,  $p_4$  being distinct prime numbers, is composed of groups which separately contain 22 proper subgroups and will be regarded as a single group in accord

with the enumeration employed above. Similarly, the triply infinite system of cyclic groups of order  $p_1 p_2^2 p_3^3$  is composed of groups which separately involve 22 proper subgroups. There is a doubly infinite system of cyclic groups whose order is of the form  $p_1^7 p_2^2$ ,  $p_1$  and  $p_2$  being distinct prime numbers which separately contain 22 proper subgroups. The direct product of the abelian group of order 8 and of type 2, 1 and the cyclic group of order  $p^3$ ,  $p$  being any odd prime number, and the direct product of the non-cyclic group of order 25 and the cyclic group of order  $p^3$ , where  $p$  is any prime number besides 5, also contain 22 proper subgroups.

The triply infinite system of cyclic groups of order  $p_1 p_2 p_3^6$ ,  $p_1$ ,  $p_2$  and  $p_3$  being distinct prime numbers, is composed of groups which separately contain exactly 22 proper subgroups. This is also the case as regards the doubly infinite system composed of the direct product of the non-cyclic group of order 9 and the cyclic group of order  $p_1 p_2$ ,  $p_1$  and  $p_2$  being any distinct prime numbers except 3. The doubly infinite system of cyclic groups of order  $p_1^3 p_2^5$ ,  $p_1$  and  $p_2$  being any distinct prime numbers, is also composed of groups involving separately 22 proper subgroups. This is also true with respect to the simply infinite system composed of the direct product of the non-cyclic group of order 9 and the cyclic group whose order is the cube of any prime number besides 3. Finally, when  $G$  is the cyclic group of order  $p_1 p_2^{11}$ ,  $p_1$  and  $p_2$  being any distinct prime numbers, it contains 22 proper subgroups. *The total number of the abelian groups which separately contain exactly twenty-two proper subgroups is therefore eleven, infinite systems of groups of the same structure being regarded as individual groups.*

If an abelian group contains exactly 23 proper subgroups and has an order which is a power of a prime number it is the cyclic group of order  $p^{24}$ ,  $p$  being an arbitrary prime number. If its order is not a power of a prime number it is the direct product of two Sylow subgroups which separately involve 5 subgroups. It is therefore either the cyclic group of order  $p_1^4 p_2^4$ ,  $p_1$  and  $p_2$  being distinct prime numbers, or the direct product of the four group and the cyclic group of order  $p^4$ ,  $p$  being any odd prime number. It was noted above that there are 7 prime power groups which separately contain exactly 24 proper subgroups, viz., the cyclic group of order  $p^{26}$ ,  $p$  being any prime number, the non-cyclic group of order  $23^2$ , the abelian group of order  $11^3$  and of type 2, 1, the abelian group of order  $7^4$  and of type 3, 1, the abelian group of order  $5^6$  and of type 4, 1, the abelian group of order  $3^7$  and of type 6, 1, and the abelian group of order  $2^9$  and of type 8, 1. If an abelian group contains 24 proper subgroups and has an order which is not a power of a prime number it is the direct product of two Sylow groups which involve 2 and 13 subgroups, respectively. Hence it is the cyclic group of order  $p_1 p_2^{12}$ ,  $p_1$  and  $p_2$  being distinct prime numbers.

It remains only to consider the abelian groups which contain exactly 25 proper subgroups. It was noted above that there are exactly two prime

power groups which have this property, viz., the cyclic group of order  $p^{26}$ ,  $p$  being any prime number, and the group of order 16 and of type 2, 1, 1. If an abelian group whose order is not a power of a prime number contains exactly 25 proper subgroups it is the direct product either of two prime power groups which separately contain 3 or 9 subgroups or of three prime power groups each of which contains three subgroups. In the former case it is the cyclic group of order  $p_1^2 p_2^8$ ,  $p_1$  and  $p_2$  being distinct prime numbers, while in the latter case it is the cyclic group of order  $p_1^2 p_2^2 p_3^2$ ,  $p_1, p_2, p_3$  being any three distinct prime numbers. Hence *there are four abelian groups which have the common property that each of them contains exactly twenty-five proper subgroups.*

## ON THE INTERSECTIONS OF IRREDUCIBLE COMPONENTS IN THE MANIFOLD OF A DIFFERENTIAL POLYNOMIAL

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1. *Formulation of Theorem.*—Let  $\Sigma$  be a system of differential polynomials in  $y_1, \dots, y_n$ . An essential irreducible manifold in the manifold of  $\Sigma$  will be called an *irreducible component* (often simply *component*) of the manifold of  $\Sigma$ .

Let  $F$  be a form in  $y_1, \dots, y_n$ . Two components of the manifold of  $F$  may have solutions in common. We are going to present a simple sufficient condition for a solution of  $F$  to belong to only a single component.

For  $i = 1, \dots, n$ , a letter  $y_{ij}$ , ( $j \geq 0$ ), will be called a *letter in  $F$*  if the order of  $F$  in  $y_i$  is at least  $j$ . We prove the following theorem.

**THEOREM:** *Let  $F$  be a form in  $y_1, \dots, y_n$ . Let*

$$\bar{y}_1, \dots, \bar{y}_n \quad (1)$$

*be a solution of  $F$ . If there is a letter  $y_{ij}$  in  $F$  such that  $\partial F / \partial y_{ij}$  is not annulled by (1), the solution (1) is contained in only a single irreducible component of the manifold of  $F$ .*

In particular, if  $F$  vanishes for  $y_i = 0$ ,  $i = 1, \dots, n$ , and, considered as a polynomial in the  $y_{ik}$ , contains at least one term of the first degree, the solution  $y_i = 0$  belongs to only one component.

2. *Proof.*—Let  $F_j$  denoted the  $j$ th derivative of  $F$ . We know that  $F$  can be decomposed into irreducible systems by choosing a sufficiently large integer  $p$  and resolving the system

$$F, F_1, \dots, F_p \quad (2)$$



considered as a set of simple forms, into indecomposable systems. We shall show that, for any  $p \geq 1$ , (2) yields only one essential indecomposable system which vanishes when each  $y_{ij}$  in (2) is replaced by  $\bar{y}_{ij}$  as determined by (1). This will prove our theorem.

Reassigning the subscripts of the  $y_i$  if necessary, let us assume that one or more  $\partial F / \partial y_{1j}$  do not vanish for (1) and let  $m$  be the greatest value of  $j$  for which vanishing does not occur. Putting the forms in (2) equal to zero, we secure a set of equations which we shall regard as equations to be solved for those  $y_{1, m+j}$  for which  $0 \leq j \leq p$ , in terms of  $x$  and the other  $y_{ik}$  in (2).

Let  $\xi$  be a value of  $x$  at which the coefficients in  $F$  and the functions in (1) are analytic and at which  $\partial F / \partial y_{1m}$  does not vanish for (1). Let  $[\eta]$  represent, collectively, the values at  $x = \xi$  of the  $\bar{y}_{ij}$  in the solution of (2) derived from (1).

The forms in (2) vanish at the point  $\xi, [\eta]$  in the space of  $x$  and the  $y_{ij}$  in (2). We shall examine, at  $\xi, [\eta]$ , the jacobian with respect to  $y_{1m}, \dots, y_{1, m+p}$  of the forms in (2). In the first row of this jacobian, which row we understand to consist of partial derivatives of  $F$ , only the first term  $\partial F / \partial y_{1m}$  fails to vanish at  $\xi, [\eta]$ . To treat the other rows, let us imagine the forms in (2) expanded in powers of the various differences  $y_{ij} - \bar{y}_{ij}$ . The expansion of  $F$  will contain a term  $\alpha(y_{1m} - \bar{y}_{1m})$  where  $\alpha$  is the function of  $x$  to which  $\partial F / \partial y_{1m}$  reduces for (1). By the nature of  $m$ ,  $F_1$  must contain the term  $\alpha(y_{1, m+1} - \bar{y}_{1, m+1})$  and can have no term  $\beta(y_{1j} - \bar{y}_{1j})$  with  $j > m+1$ . Thus, in the second row of the jacobian, the value of the second element at  $\xi, [\eta]$  is that of  $\partial F / \partial y_{1m}$  and the elements which follow have zero values. Continuing, we find the value of the jacobian at  $\xi, [\eta]$  to be the  $(p+1)$ th power of the value of  $\partial F / \partial y_{1m}$ .

Thus, for the neighborhood of  $\xi, [\eta]$ ,  $y_{1m}, \dots, y_{1, m+p}$  are determined by our equations as analytic functions  $f_m, \dots, f_{m+p}$  of  $x$  and the remaining  $y_{ij}$ . By specializing the  $y_{ij}$  in the  $f$  as functions of  $x$ , we can construct solutions of (2). Indeed, we secure in this way all solutions of (2) which, in an area contained in a small neighborhood of  $x = \xi$ , approximate closely to the solution of (2) derived from (1).

Some essential indecomposable system—call it  $\Sigma$ —in the decomposition of (2) must vanish when  $y_{1m}, \dots, y_{1, m+p}$  are replaced by their  $f$ . This  $\Sigma$  must vanish for the  $\bar{y}_{ij}$ . If an indecomposable system  $\Sigma^1$  which  $\Sigma$  does not hold vanishes for the  $\bar{y}_{ij}$ ,  $\Sigma^1$  has solutions which are not in the manifold of  $\Sigma$  and which approximate closely to the  $\bar{y}_{ij}$ . Thus, by what precedes,  $\Sigma$  is the only indecomposable system secured from (2) which has the  $\bar{y}_{ij}$  as a solution. Q. E. D.

3. *The Higher Cases.* If one allows all of the  $\partial F / \partial y_{ij}$  to vanish and requires the non-vanishing of one or more partial derivatives of the second order, there is no upper bound to the number of components to which a

solution may belong. We illustrate this by an example in the single unknown  $y$ . Let

$$F = y_2^2 + \prod_{j=1}^m [(x+j)y_1 - y]$$

where  $m$  is any integer greater than unity. Now  $(x+j)y_1 - y$  has  $(x+j)y_2$  as derivative and therefore has, for every  $j$ , a manifold which is a component of  $F$ . The solution  $y = 0$  belongs to every such component.

## DIFFERENTIALS OF FUNCTIONS WITH ARGUMENTS AND VALUES IN TOPOLOGICAL ABELIAN GROUPS<sup>1</sup>

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1. *Introduction.*—By a topological abelian group  $T$  (t.a.g.  $T$ ) we shall mean an abstract abelian group—written additively—such that (a) the function  $x + y$  and the inverse function  $-x$  are continuous functions (neighborhood continuity) of both variables  $x$  and  $y$  and of the variable  $x$ , respectively, with respect to a postulated Hausdorff topology; (b) given any  $y \in T$  and any Hausdorff neighborhood  $U$  of  $0 \in T$ , there exists a “positive integer”  $n$  such that<sup>2</sup>  $y \in nU$ .

In this note we shall give brief indications of a differential calculus for functions  $f(x)$  with  $x \in$  t.a.g.  $T_1$  and values in a t.a.g.  $T_2$ . Proofs and further developments will appear elsewhere.

The real number system has entered into the various differential calculi studied so far in one or more of three ways: (1) through the independent and dependent variables; (2) in the topology via a numerically valued metric or norm; (3) as a multiplicative domain. *The differential calculus announced in the present paper does not employ the real numbers, thus giving a new flavor to an ancient subject and its modern generalizations.*

2. *First Order Differentials.*—A first order differential  $f(x_0; \delta x)$  is defined as follows. In the definition of an  $M$ -differential<sup>3</sup> given in the paper *LTS* interpret  $T_1$  and  $T_2$  to be t.a.g. and not necessarily linear topological spaces. Then replace condition 2 (b) by the condition

$$\epsilon(x_0, x_1, nx_2) = n \epsilon(x_0, x_1, x_2)$$

for all “positive integers”  $n$ , for all  $x_1$  in some Hausdorff neighborhood of  $0 \in T_1$ , and for all  $x_2 \in T_1$ . To complete the definition add the following condition:

2 (d). *There exists a Hausdorff neighborhood  $W$  of  $0 \in T_1$  with respect to*



which the following property holds: given a Hausdorff neighborhood  $V$  of  $0 \in T_2$ , there exists a Hausdorff neighborhood  $U(V)$  of  $0 \in T_1$  such that

$$\epsilon(x_0, x_1, x_2) \in V \text{ for } x_1 \in U(V) \text{ and } x_2 \in W.$$

The following theorems have been proved.

**THEOREM I.** Let  $f(x)$  be a function with values in a t.a.g.  $T_2$  and defined on a Hausdorff neighborhood of  $x_0 \in$  t.a.g.  $T_1$ . If  $f(x)$  has a first order differential  $f(x_0; \delta x)$  at  $x = x_0$ , then  $f(x_0; \delta x)$  is unique<sup>4</sup> for all  $\delta x \in T_1$ .

**COROLLARY.** If  $f(x)$  has a first order differential at  $x = x_0$ , then  $f(x)$  is continuous at  $x = x_0$ .

**THEOREM II.** If  $f_1(x)$  and  $f_2(x)$  have first order differentials at  $x = x_0$ , then  $f_3(x) = \pm n_1 f_1(x) \pm n_2 f_2(x)$  ( $n_1$  and  $n_2$  positive integers) has a first order differential at  $x = x_0$  given by

$$f_3(x_0; \delta x) = \pm n_1 f_1(x_0; \delta x) \pm n_2 f_2(x_0; \delta x).$$

**THEOREM III.** Let  $T_1, T_2, T_3$  be t.a.g., not necessarily distinct, and  $U_{x_0}$  a Hausdorff neighborhood of  $x_0 \in T_1$ . If  $f(x)$  on  $U_{x_0}$  to  $T_2$  has a first order differential at  $x = x_0$  and if  $\phi(y)$  on  $f(U_{x_0})$  to  $T_3$  has a first order differential at  $y_0 = f(x_0)$ , then  $\psi(x) = \phi(f(x))$  has a first order differential at  $x = x_0$  given by

$$\psi(x_0; \delta x) = \phi(f(x_0); f(x_0; \delta x)).$$

**THEOREM IV.** The property of first order differentiability of a function with arguments and values in topological abelian groups is invariant under topological isomorphisms of the topological abelian groups. In particular, the invariance<sup>5</sup> is maintained under a passage to equivalent Hausdorff topologies of the topological abelian groups.

**THEOREM V.** If the topological abelian groups  $T_1$  and  $T_2$  are linear topological spaces and if  $f(x)$  has a first order differential at  $x = x_0 \in T_1$ , then the  $M$ -differential of  $f(x)$  at  $x = x_0$  exists and the two differentials are equal. The validity of the converse statement is an open question.

**THEOREM VI.** If the topological abelian groups  $T_1$  and  $T_2$  are complete normed linear spaces (Banach spaces) and if  $f(x)$  has a first order differential at  $x = x_0 \in T_1$ , then the Fréchet differential of  $f(x)$  at  $x = x_0$  exists and the two differentials are equal.<sup>6</sup> Conversely if  $f(x)$  has a Fréchet differential at  $x = x_0$ , then a first order differential (in our sense) of  $f(x)$  at  $x = x_0$  exists and the two differentials are equal.

We remark here that if we dispense with condition 2 (c) in the definition of a first order differential, then all the above theorems except Theorem V continue to hold.

3. *Second Order Differentials.*— $n$ th successive first order differentials can be defined inductively whenever the  $(n - 1)$ st successive first order differential exists for  $x$  in a neighborhood of an element  $x_0$ . In this section,

however, we shall turn our attention to an inductive definition of  $n$ th order differentials at  $x = x_0$  by assuming the existence of the  $(n - 1)$ st order differentials and the preceding differentials merely at the element  $x = x_0$ . For the purposes of our brief exposition, we shall do this here only for second order differentials.

Let  $T_1$  be a t.a.g. and  $T_2$  a t.a.g. with 0 as the only element of finite order. Let  $f(x)$  possess a first order differential  $f(x_0; \delta x)$  at  $x = x_0$ . A function  $f(x_0; \delta_1 x; \delta_2 x)$  will be called a second order differential of  $f(x)$  at  $x = x_0$  with increments  $\delta_1 x$  and  $\delta_2 x$ , if

- (1)  $f(x_0; \delta_1 x; \delta_2 x)$  is a 2-uniform symmetric bilinear function;
- (2) there exists a function  $\epsilon(x_0, x_1, x_2, x_3)$  with arguments in  $T_1$  and values in  $T_2$  such that
  - (a)  $\epsilon(x_0, 0, x_2, x_3) = 0$  for all  $x_2, x_3 \in T_1$ ,
  - (b)  $\epsilon(x_0, x_1, nx_2, mx_3) = nm \epsilon(x_0, x_1, x_2, x_3)$  for all "positive integers"  $n$  and  $m$ , for all  $x_1$  in some Hausdorff neighborhood of  $0 \in T_1$ , and for all  $x_2, x_3 \in T_1$ ,
  - (c)  $\epsilon(x_0, x_1, x_2, x_3)$  is continuous in  $(x_1, x_2, x_3)$  at  $x_1 = 0, x_2 = x_2, x_3 = x_3$  for all  $x_2, x_3 \in T_1$ ,
  - (d) there exist neighborhoods  $W_1$  and  $W_2$  of  $0 \in T_1$  with respect to which the following property holds: given a neighborhood  $V$  of  $0 \in T_2$ , there exists a neighborhood  $U(V)$  of  $0 \in T_1$  such that  $\epsilon(x_0, x_1, x_2, x_3) \in V$  for  $x_1 \in U(V), x_2 \in W_1, x_3 \in W_2$ ;

- (3) there exists some neighborhood  $N$  of  $0 \in T_1$  such that for all  $\delta x \in N$ ,  $f(x_0; \delta_1 x; \delta_2 x)$  is a second order approximation to the difference  $f(x_0 + \delta x) - f(x_0)$  in the sense that  $2[f(x_0 + \delta x) - f(x_0) - f(x_0; \delta x)] - f(x_0; \delta x; \delta x) = \epsilon(x_0, \delta x, \delta x, \delta x)$  for all  $\delta x \in N$ .

**THEOREM VII.** *If a second order differential  $f(x_0; \delta_1 x; \delta_2 x)$  of  $f(x)$  exists at  $x = x_0$ , then it is unique<sup>a</sup> for all  $\delta_1 x, \delta_2 x \in T_1$ .*

**THEOREM VIII.** *If  $f_1(x)$  and  $f_2(x)$  possess second order differentials at  $x = x_0$ , then the second order differential of  $\pm n_1 f_1(x) \pm n_2 f_2(x)$  exists at  $x = x_0$  and is given by  $\pm n_1 f_1(x_0; \delta_1 x; \delta_2 x) \pm n_2 f_2(x_0; \delta_1 x; \delta_2 x)$ .*

**THEOREM IX.** *Let  $T_1$  be a t.a.g., and  $T_2$  and  $T_3$  two t.a.g. without elements of finite order other than their 0 elements, and let  $U_{x_0}$  be a Hausdorff neighborhood of  $x_0 \in T_1$ . If  $f(x)$  on  $U_{x_0}$  to  $T_2$  possesses a second order differential at  $x = x_0$  and if  $\phi(y)$  on  $f(U_{x_0})$  to  $T_3$  possesses a second order differential at  $y_0 = f(x_0)$ , then  $\psi(x) = \phi(f(x))$  possesses a second order differential at  $x = x_0$  given by the formula*

$$\psi(x_0; \delta_1 x; \delta_2 x) = \phi(f(x_0); f(x_0; \delta_1 x); f(x_0; \delta_2 x)) + \phi(f(x_0); f(x_0; \delta_1 x; \delta_2 x)).$$

**COROLLARY.** *The correspondent of Theorem IV for second order differentiability of a function.*

In conclusion, we wish to remark that with the aid of the concept of a

product topological space it is possible to treat total differentials of functions of several t.a.g. variables.

<sup>1</sup> The results on first order differentials were presented to the American Math. Society at the Pasadena meeting, Dec. 2, 1939.

<sup>2</sup> By  $nx$  we understand the group sum  $x + x + \dots + x$  with  $n$  summands. Similarly  $-nx$  will stand for the group difference  $-x - x - \dots - x$ . If  $S \subseteq T$ , then by  $nS$  we mean the set of all elements  $nx$  with  $x \in S$ . Clearly, condition (b) in the definition of a t.a.g.  $T$  becomes redundant whenever  $T$  is specialized to be a linear topological space with real number multipliers. It is of interest to note here that throughout the whole paper, the Hausdorff separation axiom can be replaced by the weaker Fréchet separation axiom.

<sup>3</sup> Michal, A. D., "Differential Calculus in Linear Topological Spaces," these PROCEEDINGS, 24, 340-342 (1938). The abbreviation *LTS* will be used to refer to this paper. See also Michal, A. D., "General Differential Geometries and Related Topics," *Bull. Amer. Math. Soc.*, 45, 529-563 (1939).

<sup>4</sup> Condition (b) in the definition of a topological abelian group is used in this paper only to prove the uniqueness of the differentials for all values of the increment. Moreover the uniqueness Theorem I continues to hold even if  $T_2$  does not satisfy condition (b). This makes possible a treatment of differentials of set-valued functions of a t.a.g. variable. We also plan to study differentials of functions of point set variables.

<sup>5</sup> If  $T_1$  and  $T_2$  are Banach spaces, then Fréchet differentiability is invariant only under a passage to equivalent Banach topologies of  $T_1$  and  $T_2$  whereas our differentiability is invariant under a passage to equivalent Hausdorff topologies of  $T_1$  and  $T_2$ .

<sup>6</sup> We treat briefly polynomials and their polars with t.a.g. variables and then apply them to the proof of Theorem VII and its extensions.

## TRIANGULATED MANIFOLDS WHICH ARE NOT BROUWER MANIFOLDS

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The following results, which will be elaborated elsewhere, grew from a study of Brouwer's definition<sup>1</sup> of a manifold, in its connection with questions of differentiability, analyticity and polyhedral imbedding. We first enumerate several definitions:

(1) *Topological  $m$ -manifold*: A connected topological space which can be covered by a denumerable set of neighborhoods, each of which is an  $m$ -cell.

(2) *Triangulable manifold*: A topological manifold which can be subdivided into the cells of a complex.

(3) *Star  $m$ -manifold*: A triangulated  $m$ -manifold on which the region covered by the star of any vertex is an  $m$ -cell.

(4) *Brouwer  $m$ -manifold*:<sup>1</sup> A star  $m$ -manifold for which the star of each vertex can be mapped homeomorphically into a euclidean  $m$ -space,  $E^m$ , so that the image of each  $m$ -cell of the star is an  $m$ -simplex.

(5) *Differentiable [analytic]  $m$ -manifold*: A topological  $m$ -manifold which can be covered with local coördinate systems having  $m$ -cells for domains, such that the transformation between any two of the systems on the intersection of their domains is differentiable [analytic] with a non-vanishing jacobian.

A few results can be stated in partial answer to the general question whether any two of the above classes of manifolds are topologically equivalent in the sense that an arbitrary member of either class has a homeomorphic image in the other. For  $m < 3$ , all the classes are known to be equivalent. Whitney's work<sup>2</sup> shows that differentiable and analytic manifolds are topologically equivalent. In a forthcoming paper,<sup>3</sup> the writer shows that the classes numbered (2) to (5) are all equivalent for  $m = 3$ . Each of the classes, as arranged above, is topologically equivalent to a subset of each preceding class.<sup>4</sup> The following theorem is new.

(A) *For every  $m > 3$ , there exist star  $m$ -manifolds which are not Brouwer manifolds. This is equivalent to saying that there exists, for every  $m > 3$ , a star of simplexes which is an  $m$ -cell but cannot be mapped into an  $E^m$  by a homeomorphism linear on each simplex of the star.*

This result is but one of the implications of a triangulation,  $\tau$ , of an  $(m-1)$ -simplex,  $s$  ( $m = 4, 5, \dots$ ), constructed by the writer in such a fashion that  $\tau$  is not homeomorphic to any rectilinear triangulation of  $s$ . Other results depending on the existence of such a triangulation are stated below.

By a *polyhedral representation* of a triangulated manifold, we mean the image of the manifold in a euclidean space under a homeomorphism which sets each cell of the triangulation into correspondence with a euclidean simplex. The following theorems then hold.

(B) *Every triangulated  $m$ -sphere,  $m < 3$ , has a convex polyhedral representation in  $E^{m+1}$ . For every  $m \geq 3$ , there exist triangulated  $m$ -spheres which have no convex polyhedral representations in  $E^{m+1}$ .*

(C) *Given a finite simplicial complex,  $K$ , let  $n$  be the smallest number such that  $K$  has a polyhedral representation in  $E^n$ . Then  $n$  is not, for every  $K$ , invariant under subdivisions. In some cases,  $n$  can be made alternately to increase and decrease during a sequence of successive subdivisions.*

A polyhedral  $m$ -manifold,  $P^m$ , in  $E^n$  is said to be in *normal position*, if there exists a continuously varying transversal  $(n-m)$ -plane<sup>5</sup>  $\pi^{n-m}(p)$  as  $p$  ranges over  $P^m$ . A Brouwer  $m$ -manifold,  $M$ , is characterized by the fact that it has a polyhedral representation,  $P^m$ , in an  $E^n$ , such that transversal  $(n-m)$ -planes exist at each vertex. The writer has partly treated (see *H*) the question of the possibility of extending the definition of these transversal planes over the whole of  $P^m$ , thus showing it to be in normal position.

If and only if this can be done, it is possible (see *H*) to apply Whitney's method<sup>2</sup> of constructing an analytic approximation to  $P^m$  and hence a homeomorphic image of  $M$  in the class of analytic manifolds. Hence part of the significance of the following statement.

(*D*) *The star manifolds of Theorem (A) above do not admit polyhedral representations in normal position. For every  $m > 3$ , there exist stars of simplexes which are  $m$ -cells but do not admit transversal  $(n - m)$ -planes no matter how they are polyhedrally represented in any  $E^n$ .*

It is uncertain whether, by changing the triangulation, these same star manifolds can always be made into Brouwer manifolds. If not, then there exist manifolds which cannot be made differentiable.<sup>4</sup>

(*E*) *Brouwer's definition<sup>1</sup> of an  $m$ -manifold is not invariant ( $m > 3$ ) under subdivisions.*

Some of the above results have interesting interpretations in the space,  $\Pi$ , of  $(n - m)$ -planes through a point,  $O$ , in  $E^n$ . A subspace of  $\Pi$  is the space  $\Pi(S)$  of planes transversal to a given star,  $S$ , of  $m$ -simplexes incident with  $O$ . The space  $\Pi$  can be defined with the aid of Vahlen's relations among the determinants of a matrix, and  $\Pi(S)$  is then definable by supplementary conditions on these determinants. For the cases where  $S$  admits no transversal  $(n - m)$ -plane, we have the algebraic result that the system of equations and inequalities defining  $\Pi(S)$  is inconsistent.

<sup>1</sup> L. E. J. Brouwer, "Über Abbildungen von Mannigfaltigkeiten," *Math. Ann.*, **71**, 97-115 (1912).

<sup>2</sup> Hassler Whitney, "Differentiable Manifolds," *Ann. Math.* **37**, 645-680 (1936).

<sup>3</sup> S. S. Cairns, "Homeomorphisms between Topological Manifolds and Analytic Manifolds," to appear in the *Ann. Math.* This paper is hereafter referred to as *H*.

<sup>4</sup> For every differentiable manifold can be triangulated into a Brouwer manifold. This follows, with the aid of Whitney's imbedding theorem (*loc. cit.*), from certain results obtained by the writer. See S. S. Cairns, "Polyhedral Approximations to Regular Loci," *Ann. Math.* **37**, 409-415 (1936).

<sup>5</sup> Such a plane is characterized by the conditions that it pass through  $p$  and make angles bounded away from zero with the secants of a neighborhood of  $p$  on  $P^m$ .





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*MUTATIONS AND REVERSIONS IN REPRODUCTIVITY OF  
ASPERGILLI WITH NITRITE, COLCHICINE AND d-LYSINE*

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Read before the Academy, April 23, 1940

Claims for the chemical production of mutants in species of fungi have existed in the literature for many years.<sup>2,6</sup> The major obstacle to their acceptance has been the rarity with which positive results were obtained, hence the failure of subsequent workers in reproducing them. Under these circumstances the mutations described might be considered to have occurred without any specific relation to the experimental conditions set up. The most striking evidence for chemical induction of mutation has been obtained with colchicine in the production of polyploidy in phanerogams,<sup>1</sup> but so far all investigators, including ourselves, have reported negative results with this alkaloid on fungi.

Our first successful use of chemical induction of mutation with fungi was by means of sodium nitrite in acid solution, i.e., nitrous acid. "Injury" mutants in which differentiation, and therefore reproduction, was suppressed in varying degree were readily formed by the action of nitrite. Ease of production and duplication of results with the *Aspergilli* approached those of a chemical reaction. Positive results were obtained with selected strains of *Aspergillus niger* and *A. Amsterdami*;<sup>4</sup> and were later extended to include *A. varicolor*, *A. fumigatus*, *A. fischeri*, *A. flavus*, *A. alliaceus* and *A. nidulans*.<sup>3</sup> Though the propriety of referring to these artificially produced forms as mutants may be questioned, some evidence was obtained that they persist through the ascosporic stage.

Since the regularity and ease with which mutants were induced by nitrite suggested the smoothness of action of a chemical process, the suggestion was made that the effect of this compound consisted in the destruction of a varying number of free amino groups in proteins included in the hereditary mechanism. Polyploidy, it was considered, was contradicted by the type of mutants obtained, nor was it believed to explain the uniformity in response to treatment.

We have attempted to submit the hypothesis of amino group destruction to test. Present difficulties prevent cytological studies to ascertain the effect of the nitrite process upon the nuclear mechanism. The fact that these forms are unsuitable for such studies is not the least of these difficulties. Determination of the amino nitrogen content of the fungus proteins might be feasible but also could not be undertaken under present conditions. It seemed probable, however, that if this hypothesis had any basis in fact, the use of other compounds suitable for removal of amino groups should also lead to formation of mutants of the same type.

The compounds selected for trial included ninhydrin, chloramin T., potassium iodide and hexamethylenamine (formin). All are stable in neutral solution but become reactive under the influence of the acidity accompanying growth. The last compound was included because formaldehyde is gradually liberated from it by the steadily increasing acidity in the cultures and would be capable of combining with free amino groups of the fungus proteins. All these compounds gave a sterility type mutant with *A. niger*, the only organism investigated in this respect.

Another test of the hypothesis consisted in an attempt to reverse the results of the process of nitrite treatment. The possibility existed that replacement of amino groups in the proteins of the mutants would, if feasible, lead to reversion of mutants toward the original strains. Two conditions seemed favorable for inducing this reverse mutation. First, the use of high concentrations of d-lysine in the expectation that in some instances the added amino acid would be introduced intact into the modified proteins during their synthesis. This amino acid was chosen because free amino groups in proteins are known to be associated with the presence of d-lysine. Second, the use of solutions of neutral or slightly alkaline reaction containing ammonium and strong reducing agents, on the chance that chemical conditions favorable for the formation of amino acids from corresponding hydroxy or keto acids would promote recovery to the original strain. The organisms tested in this manner included *A. niger* and *A. Amstelodami*. Reversion mutants showing partial to complete recovery in reproductivity were obtained with *A. niger* using d-lysine or sodium thiosulfate; whereas *A. Amstelodami* as yet has given forms showing only recovery of both conidia and ascospores, but of quite different colony appearance. In both instances an excess of calcium carbonate was introduced into the culture medium.

We have at last succeeded in obtaining positive results with fungi through the use of colchicine. The explanation of the many unsuccessful attempts to obtain mutants of fungi with colchicine is believed to have a simple chemical basis. Colchicine is hydrolyzed by acid to form colchicein, which is apparently of little or no effect. The use of acid nutrient solutions, or the free acids formed through metabolism of fungi, would

serve to nullify the presence of this alkaloid. Addition of excess calcium carbonate to the nutrient solution prevents destruction of colchicine apparently, since mutants were readily obtained with *A. fumigatus*, *A. nidulans*, *A. flavus*, *A. fischeri*, *A. varicolor* and *A. alliaceus*. No mutants were obtained with *A. Amstelodami*, or *A. niger*, though indications of a positive action were given by these species also. Concentrations of colchicine greater than one per cent may be found necessary with the latter species, therefore. Whether disturbances in metabolism because of a fixed neutrality are contributory factors remains to be determined.

The types of mutants obtained with colchicine included forms similar to those obtained with nitrite. Intermediate forms were more plentiful, however, and a few forms showing increased fruitfulness were also found. Partial reversion of mutation in *A. Amstelodami* has also been accomplished with colchicine.

The changes in inheritance brought about experimentally in the eight species of Aspergilli with which we have worked are departures from the normal inheritance of these forms that have in some instances proved stable over more than a score of years. These artificially produced strains again could be duplicated in large measure from a collection of forms from natural sources geographically widely distributed and covering a collecting period of more than thirty years. Conidia and ascospores, moreover, if produced at all in these mutants, persisted unchanged from those of the original strains in form and markings. The chemical facts may also be briefly summarized. The action of nitrite results in the formation of mutants in which the ability to differentiate, and therefore to reproduce, are injured in varying degree. Other reagents known to be destructive of the amino group in amino acids and proteins have a similar effect. The presence of high concentrations of d-lysine, the amino acid presumably affected by nitrite, brings about a reversal in mutation. Chemical conditions conducive to re-introduction of amino groups also bring about a reverse mutation.

Nevertheless, the precise nature of the underlying process is by no means certain as yet in the absence of cytological and analytical data. Nor is the formation of similar type mutants by colchicine particularly helpful to the interpretation of amino group destruction. The mode of action of colchicine on fungi itself needs elucidation, since polyploidy need not necessarily or entirely enter into the observed responses with fungi.

It must be considered that nitrite and other destructive agencies acting on amino groups need not necessarily be the only means of bringing about the formation of this type of mutation. Presumably conditions may be brought about under which the metabolic introduction of ammonia to form amino groups is hindered to a greater or less extent.

Specific mention might be made in conclusion of an important implica-

tion that follows from this interpretation of the mechanism of nitrite mutation and of reversion by d-lysine. An important, if not most important rôle is here assigned to d-lysine in the process of differentiation and reproduction, and concomitantly as a possible basis for strain differences in fruitfulness and certain growth characteristics. Progressive increase in sterility in successive transfers of fungi may, in some instances, have its origin in this process, and may be found possible of counteraction in the presence of excess calcium carbonate by means of d-lysine or of ammonium salt and thiosulfate.

<sup>1</sup> Blakeslee, A. F., and Avery, A. G., *Jour. Heredity*, 28, 393-411 (1937).

<sup>2</sup> Schiemann, E., *Zeits.-Induct. Abstammungs. u. Vererbungslehre*, 8, 1-35 (1912).

<sup>3</sup> Steinberg, R. A., and Thom, C., *Jour. Heredity*, 31, 61-63 (1940).

<sup>4</sup> Thom, C., and Steinberg, R. A., *Proc. Nat. Acad. Sci.*, 25, 329-335 (1939).

<sup>5</sup> Waterman, H. J., *Zeits. Gaerungs. Physiol.*, 3, 1-14 (1913).

## A COMPARISON OF CHROMOSOMAL ABERRATIONS INDUCED BY X-RAY AND ULTRA-VIOLET RADIATION

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The investigations of Stadler and Sprague<sup>1</sup> on maize, following that of earlier workers,<sup>2,3</sup> have confirmed and furthered our knowledge as to the genetical effects of ultra-violet radiation. They showed that like x-rays, ultra-violet radiation could materially increase the mutation rate, but that on the contrary it differed from x-rays in that no increase in the frequency of translocations was found. Besides point mutations affecting seed and seedling characters, numerous entire and fractional endosperm deficiencies were found, being similar in nature, although differing in relative frequency, from those produced by x-rays. Later Singleton,<sup>4</sup> and Singleton and Clark,<sup>5</sup> showed that plants with defective pollen segregations frequently revealed deficiencies that were chromosomal in nature and not simply gene mutations. These deficiencies were cytologically demonstrable at pachytene, and invariably involved the terminal deletion of a chromosome segment. Muller and MacKensie<sup>6</sup> confirmed the results of Stadler and Sprague in so far as translocations were concerned.

The accumulated evidence to date therefore points to a qualitative difference existing between the effects of ultra-violet and x-ray radiation. In all previous ultra-violet experiments, however, examination of individuals for chromosomal changes was made only after many cell generations had intervened since the time of treatment. A direct comparison of the results

of ultra-violet and x-ray radiation, i.e., examination before the intervention of a cell generation between the time of irradiation and that of observations, might serve to settle the question of whether or not a truly qualitative difference does exist between the effects resulting from these two kinds of radiant energy. It is with this purpose in mind that the present study has been undertaken.

A direct x-ray analysis of chromosomal aberrations is a comparatively easy matter because the extreme penetrating power of these rays permits them to reach cells whose division cycle is fairly accurately known and timed,<sup>7</sup> facilitating in this manner the collection of an extensive amount of data before the elimination of non-viable chromosome alterations. A direct analysis of the effect of ultra-violet treatment has not been too successfully carried out to date because of the drastic absorption of the ultra-violet rays by overlying tissues. However, with the development of

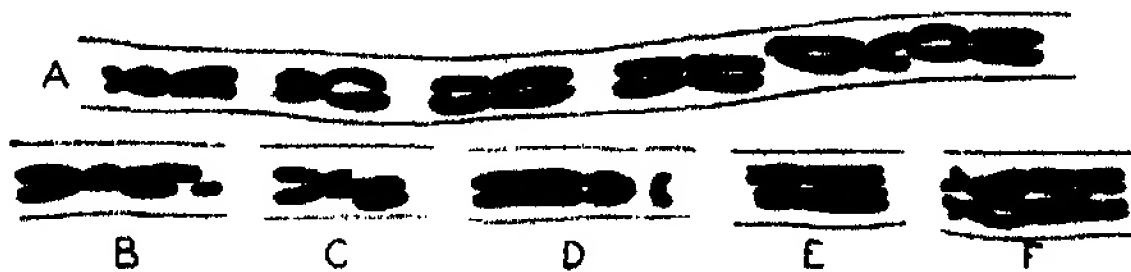


FIGURE 1

Camera lucida drawings of chromosomes in pollen tubes of *Tradescantia*. Ca. 2000  $\times$ . A. Six normal chromosomes in pollen tube. Illustrates manner in which they pass down the tube following acenaphthene treatment. B and C. Chromatid deletions induced by ultra-violet radiation. D. Chromatid dicentric induced by x-rays. E and F. Chromatid exchanges induced by x-rays.

methods for growing pollen tubes on cultural media,<sup>8</sup> a singularly simple yet effective means is available for carrying out a direct cytological analysis of any chromosomal aberrations or rearrangements induced by ultra-violet radiation (figure 1A). In this manner, the generative nucleus can be irradiated in the pollen tube, absorption, therefore, being at a minimum.

The plants used in this study were from a clonal line of a diploid species of *Tradescantia*. The source of the x-rays was a Coolidge tube equipped with a tungsten target. The source of the ultra-violet radiation was a Hanovia mercury arc lamp operating at 4 amperes and 110 volts d. c. The light was unfiltered, and the treating distance was 10 cm. The heating effect was reduced by the use of an electric fan.

*X-Ray Effects on Microspores.*—An extensive analysis of x-ray induced chromosomal aberrations in *Tradescantia* microspores has been carried out by Sax<sup>7</sup> and others. These induced changes fall into two readily distinguishable classes, their nature depending upon the conditions of the



chromosomes at the time of irradiation. Chromosome breaks result from treatment given when the chromosomes are in the form of single threads; chromatid breaks are induced at prophase after the chromosomes have become functionally split into two sister chromatids.

Chromosome breaks are of several kinds, depending upon the number of effectively broken chromosomes concerned. A single break generally involves the deletion of a portion of an arm, the deleted segment lying in the cytoplasm as an acentric fragment. Dicentric chromosomes, ring chromosomes and exchanges between non-homologous chromosomes are also frequently observed.

Chromatid breaks can likewise be classified as to their one-hit or two-hit nature. Single breaks may include one or both of the sister chromatids. If one is broken, this is visible as a shortened chromatid accompanied by a deleted fragment; if both are broken, lateral fusions between the broken ends of sister chromatids give a dicentric chromatid and a U-shaped fragment. Independent hits in two adjacent chromosomes may result in a chromatid exchange; if in the same chromosome, a ring will be produced.

*X-Ray Treatment of Dry Pollen Grains.*—Open flowers were given doses of 200 r, and the pollen grains germinated on a cultural medium. The data are given in table 1.

TABLE 1  
X-RAY DATA ON POLLEN GRAINS IRRADIATED DRY. 200 R

Figures represent number of chromosomes examined

NORMAL	CHROMATID DELETIONS	CHROMATID DICENTRICS	CHROMATID EXCHANGES	DICENTRIC CHROMOSOMES	TOTAL	% BREAKS
979	4	15	2	2	1002	2.29

Chromosome changes induced at this stage of pollen maturity are very similar in nature to those resulting from irradiation of early prophase nuclei in the microspores. The presence of two dicentric chromosomes indicates that the chromosomes of the generative nucleus at the time of pollen maturity are not all effectively split into sister chromatids. Dicentric chromosomes are never found when microspores are irradiated in prophase, so that their presence in the pollen tube is a valid criterion of the singleness of some of the chromosomes.

*X-Ray Treatment of Pollen Tubes.*—Pollen tubes were subjected to x-rays at approximately two hours after germination. As will be seen later, this particular time for irradiation was chosen so that a strictly comparable set of data might be obtained to test for the presence or absence of a qualitative difference between the effects of x-ray and ultra-violet radiation on chromosome breakage. The dosage was 240 r. The pollen tubes, however, were irradiated while still in the glass moist chamber in which



they were growing so that the actual dosage reaching the nuclei was somewhat lower due to partial absorption of the rays by the glass. The data are given in table 2.

TABLE 2					
X-RAY DATA ON POLLEN TUBES. 240 R					
NORMAL	CHROMATID DELETIONS	CHROMATID DICENTRICS	CHROMATID EXCHANGES	TOTAL	% BREAKS
946	29	22	5	1002	5.58

Under this treatment, chromatid deletions are more numerous than chromatid breaks (figure 1D), although both are undoubtedly the result of single quantum "hits." The high percentage of deletions is considerably above that found in x-rayed microspores whether rayed in prophase or resting stage, Sax<sup>7</sup> reporting that only five per cent of the aberrations induced at resting stage are of this type as opposed to over fifty percentage here. It is quite probable that an explanation may be sought in the spatial relationships of the sister chromatids at this time. The threads are without doubt farther apart when the generative nucleus has passed out into the tube than when it lies passively in the ungerminated pollen grain. Also a considerable tension is exerted on the chromosomes as they pass down the tube. This may be deduced from the fact that frequently the nucleus becomes separated into two or more independent groups of chromosomes, and also from the fact that occasional chromosomes are stretched almost to a breaking point as they move downward. This tension is probably set up by rapid protoplasmic streaming. Internal movements in the individual chromosomes as the result of prophase contraction may also be another factor in keeping broken ends from rejoining into their original positions once they have become separated from each other. If this be the case, we might expect that the number of deletions found in irradiated pollen tubes reflects more nearly the actual percentage of breaks induced by this treatment than does the observed percentage of breaks in the microspores, where, according to Sax,<sup>7</sup> the frequency of actual breaks undoubtedly is much greater than the observed frequency, the majority of them reuniting back into their original positions.

In addition, chromatid exchanges (figure 1E, F) between non-homologous chromosomes are also present. No dicentric chromosomes were found. Evidently completion of the effective splitting had taken place by this time, and all aberrations could be classified as chromatid breaks.

*Ultra-Violet Treatment of Pollen Tubes.*—Pollen grains were germinated on slides and the generative nucleus irradiated two hours after sowing. Exposure was for 30 seconds. Longer exposures were found to be inadvisable because the film of medium soon becomes desiccated, inhibiting

the growth of the pollen tubes and finally causing death. Three different groups of slides were analyzed (table 3).

TABLE 4  
ULTRA-VIOLET DATA ON POLLEN TUBES  
30-second exposures at distance of 10 cm.

NORMAL	CHROMATID DELETIONS	CHROMATID DICENTRICS	TOTAL	% BREAKS
(a) 2155	23	0	2178	1.05
(b) 1273	11	0	1284	0.86
(c) 1565	11	2	1578	0.82
Con. 1587	2	1	1590	0.18

Experimental evidence reveals that the chromosomal aberrations induced by ultra-violet radiation are almost exclusively of the chromatid deletion type (figure 1*B, C*). So far as could be determined cytologically all deletions were terminal in nature, and of varying length, the breaks not being localized in any particular regions. All gradations from free fragments to achromatic lesions were found. However, only those breaks which unquestionably showed broken ends were considered in this study, although some of the achromatic lesions were undoubtedly true deletions which had not as yet become separated from the remainder of the chromosome. No lesions were found that extended across both chromatids at the same locus.

Only two chromatid dicentrics were observed in the ultra-violet treated material (figure 1*D*). Unlike the deletions, these aberrations involve breakage of both chromatids at the same locus, followed by lateral fusion to give the dicentric chromatid plus the U-shaped fragment. One of the two breaks lacked the usual fragment, and an examination of the entire length of the pollen tube and grain failed to reveal it lying in the cytoplasm. Evidently the above dicentric chromatid was the result of some previous microspore aberration which had been carried over into the generative nucleus. The only other chromatid break was similar to that found in the control, and probably was of spontaneous origin.

*Discussion.*—It has thus been possible to obtain a direct analysis of ultra-violet induced chromosomal aberrations, confirming in this manner the genetical results of Stadler and Sprague<sup>1</sup> and of Muller and MacKensie.<sup>6</sup> Cytologically observable deficiencies occur, but no increase in the percentage of translocations was found. The fact that a single effective ultra-violet "hit" can only break a single thread at any one locus while a single x-ray "hit" can break both threads seems best explained by assuming that the sphere of influence of an absorbed ultra-violet quantum is an area no greater in diameter than the width of a single chromatid, while that of an x-ray quantum is sufficiently wide in extent to include both. On the basis of energy values this becomes explicable, for as Good-

speed and Uber<sup>9</sup> point out, the energy of an ultra-violet quantum is from 4 to 6 electron-volts, whereas that of an x-ray quantum may range from 10,000 to 200,000 or more, the value increasing inversely as the wavelength.

The difference in the width of this so-called "sphere of influence" may perhaps be thought of as an expression of the difference in the physical behavior attending the absorption of these quanta. The curves obtained when breaks<sup>7</sup> or survival ratios<sup>10, 11</sup> are plotted against dosage imply the effectiveness of single quantum absorptions. It is now well established that the ionization created by x-rays is responsible for the chromosome breakage. Ultra-violet, on the other hand, because of the low energy values of a single quantum, cannot create a path of intense ionization. Instead, it derives its effectiveness from the fact that it can excite the absorbing molecules to higher energy states by affecting the electrons in only the outer orbits. Ultra-violet has, on this account, been frequently spoken of as "chemical rays." This ionization is therefore highly localized, and does not extend across the distance between the sister chromatids to cause a similar reaction at the same locus in the other thread.

This, however, does not explain the absence of translocations under ultra-violet treatment. The question arises as to whether or not chromosome breaks induced by ultra-violet radiation are capable of reuniting back into their original positions or into new associations. Sax<sup>12</sup> has shown that x-ray induced breaks can remain in an unstable condition and capable of re-fusion for as long as an hour after the time of irradiation. Does the absorption of an ultra-violet quantum leave a broken end in such a labile condition, or does the chemical action of these rays leave a satisfied bond at the broken end such that re-fusion is impossible? For the present this question must remain unanswered, but its solution might serve to explain the absence of gross chromosomal rearrangements with ultra-violet treatment under circumstances where comparable doses of x-ray, as judged from the frequency of endosperm deficiencies<sup>1</sup> and lethals,<sup>6</sup> produce an abundance of these types of aberrations.

Ultra-violet radiation of maize pollen<sup>1</sup> yields two kinds of endosperm deficiencies, entire and fractional, as judged by the absence of dominant endosperm characters. The fractional deficiencies generally consist of kernels showing 1/2 of the tissue recessive and the other 1/2 dominant, with about equal numbers of larger and smaller proportions. If we assume that the aberration induced by the treatment consists of the deletion of a segment of the chromosome arm when the chromosome is in the two-thread state, the production of fractionals becomes readily understandable. This is indicated by Stadler and Sprague's data which show that losses of *C* and *Wx* are usually associated, eliminating the possibility that these are simple point mutations. The first division of the endosperm-

fusion-nucleus, resulting from fertilization by such an altered sperm cell, would therefore give one cell with a normal, and one with a deleted, chromosome. The amount of endosperm derived from each of these cells would be approximately equal, giving kernels chiefly of the "1/2" class, but if the products of the two cells were unequal, as well might be the case since division in the endosperm is an irregular process, then equal numbers of larger and smaller proportions would be expected.

The production of entire endosperm deficiencies by ultra-violet treatment is not so easily explained. There are four possible ways by which this might be brought about: (1) ultra-violet induces breaks in both chromatids of a single chromosome arm by two independent absorptions, although on a random basis this seems very unlikely if all breaks are simple deletions; (2) a single "hit" can induce a chromatid break involving both chromatids at the same locus, a situation not yet demonstrated cytologically; (3) a single quantum might be absorbed in the region of the centromere where the thread is effectively single although the chromatids are split, giving a deletion for both of the chromatids of an arm; (4) if all of the chromosomes in the sperm nuclei or generative nucleus are not effectively split at the time of irradiation an opportunity is provided for a single "hit" to delete the entire end of a chromosome arm, thus giving an endosperm-fusion-nucleus deficient for that particular segment. At present this explanation seems to be the most satisfactory, and is not without some experimental evidence, for, as has been pointed out above, the appearance of dicentric chromosomes in pollen grains irradiated dry is good factual evidence for the presence of single threads at the time of irradiation. Furthermore, in maize, where division of the generative nucleus to give two sperm cells takes place in the grain instead of in the pollen tube as in *Tradescantia*, the possibility of having single threads in the sperm nuclei is obviously greater than in the generative nucleus of *Tradescantia* since no further division of the sperm nuclei takes place before fertilization.

X-ray induced endosperm deficiencies<sup>1</sup> are generally of the "entire" kind, while those induced by ultra-violet are largely fractionals. The experimental data obtained in this study are in good agreement with these conclusions since the chromatid deletions resulting from ultra-violet radiation would give genetically observable fractional deficiencies while the greater proportion of chromatid dicentrics induced by x-rays would result in entire endosperm deficiencies.

*Summary.*—X-ray radiation of the generative nucleus in *Tradescantia* pollen grains reveals that most of the chromosomes are effectively split into sister chromatids. A great proportion of the chromatid aberrations involve deletions of both chromatids of a chromosome at identical loci, thus confirming the genetic data in maize in respect to endosperm deficiencies. Irradiation of the generative nucleus in the pollen tube results

in only chromatid aberrations including a considerable number of simple chromatid deletions and occasional chromatid exchanges

Ultra-violet radiation of the generative nucleus in the pollen tube induces only simple chromatid deletions. The loss of only one of the two chromatids is in accord with the genetic observations that ultra-violet radiation produces primarily fractional endosperm deficiencies in maize. No configurations representing an interchange of chromatin between non-homologous chromosomes were found.

The qualitative difference between the types of breaks induced by x-ray and ultra-violet radiation is tentatively explained by assuming that the sphere of influence of a single x-ray quantum is much greater in area than that of a single ultra-violet quantum. The vast difference in energy values, and the difference in the physical behavior attendant to absorption of the respective quanta supply a possible physical and chemical basis for this variation in degree of effectiveness.

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## CYTOLOGY AND DEVELOPMENT OF THE EMBRYOS OF X-RAYED ADULT *DROSOPHILA MELANOGASTER*

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In 1927 Muller<sup>1</sup> conclusively demonstrated that mutations could be induced in animals by means of x-rays. Exposure of the germ cells of *Drosophila melanogaster* to relatively heavy doses of x-rays resulted in the production of large numbers of mutations of different types, including recessive lethals, semi-lethals and visibles of various kinds. "In addition, it was also possible to obtain evidence in these experiments for the first time, of the occurrence of dominant lethal genetic changes, both in the X



and in the other chromosomes. Since the zygotes receiving these never developed to maturity, such lethals could not be detected individually, but their number was so great that through egg counts and effects on the sex ratio evidence could be obtained of them en masse." Dominant lethals are, thus, a class of lethals which can effect the death of zygotes even though present in single dose, i.e., introduced by but one of the gametes.

Hanson,<sup>2</sup> shortly thereafter, subjected *Drosophila* males to x-rays and made counts to determine the extent of the effects of the radiations. Mortality of the  $F_1$  eggs, larvae and pupae when compared to the death rate among the controls was found to be significantly increased. He inferred that dominant lethal mutations had been induced in the treated sperm, resulting in death of the zygotes at various stages in their ontogeny. Timofeef-Ressovsky,<sup>3</sup> Gowen and Gay,<sup>4</sup> Sonnenblick,<sup>5</sup> Demerec, Kaufmann and Hoover,<sup>6</sup> Kaufmann<sup>7</sup> and others have also noted the marked increase in mortality among the progeny of irradiated adult *Drosophila*. Furthermore, the induction of non-transmissible dominant lethals in *Habrobracon* has been reported.<sup>8, 9</sup>

Although the progeny of treated *Drosophila* die at various levels of development, the high death rate among the embryos is especially striking. In those zygotes which initially commenced development with one haploid complement of untreated chromosomes and with one irradiated chromosomal complement, the majority of the inviable alterations and rearrangements introduced by the treated gametes are thus eliminated prior to hatching of the young larvae. This report, based on egg counts and the study of preparations of approximately one thousand eggs and embryos, will indicate the nature of the inviable chromosomal alterations and their effects on embryonic development.

A wild type Oregon-R strain of *Drosophila melanogaster* was used in the experiments. Tests with this vigorous stock indicate that more than 96 per cent of the deposited normal eggs hatch as larvae. But 55 of 1420 zygotes deposited by untreated parents failed to emerge from the embryonic envelopes. Five-day-old virgins were employed throughout all of the work. The flies were put into small gelatin capsules which were placed on a sheet of cardboard at a distance of 34 cm. from the tungsten target. The tube was operated at 200 kv. and at 30 ma. current. Under these conditions, the output of the machine, as measured in air, was 390 r per minute. Dosages ranging from 195 r to 4680 r were used. The various exposures were given by stopping the machine at measured intervals and removing the samples in sequence. Males and females, in separate capsules, were simultaneously subjected to the radiations and, following the exposure, mated to untreated virgins. Eggs were collected on the moist inside surface of ripe banana skins and at timed periods of development



were fixed and sectioned, securing organisms which were representative of all levels of embryonic development for comparison with controls.

I am greatly indebted to Dr. Paul S. Henshaw, formerly of the Memorial Hospital, New York City, and now at the National Cancer Institute, Washington, D. C., for the x-ray treatments.

*Results: (a) Egg Mortality Counts.*—The testes of adult males carry an abundant supply of sperm<sup>10</sup> which are passed to the mates during copulation. Thus, radiation of the males involves exposure of gametes which had already undergone maturation. In the females, however, the situation is different. The physiological condition of the chromosomes at the time of exposure is not like that obtaining in the males. With the ex-

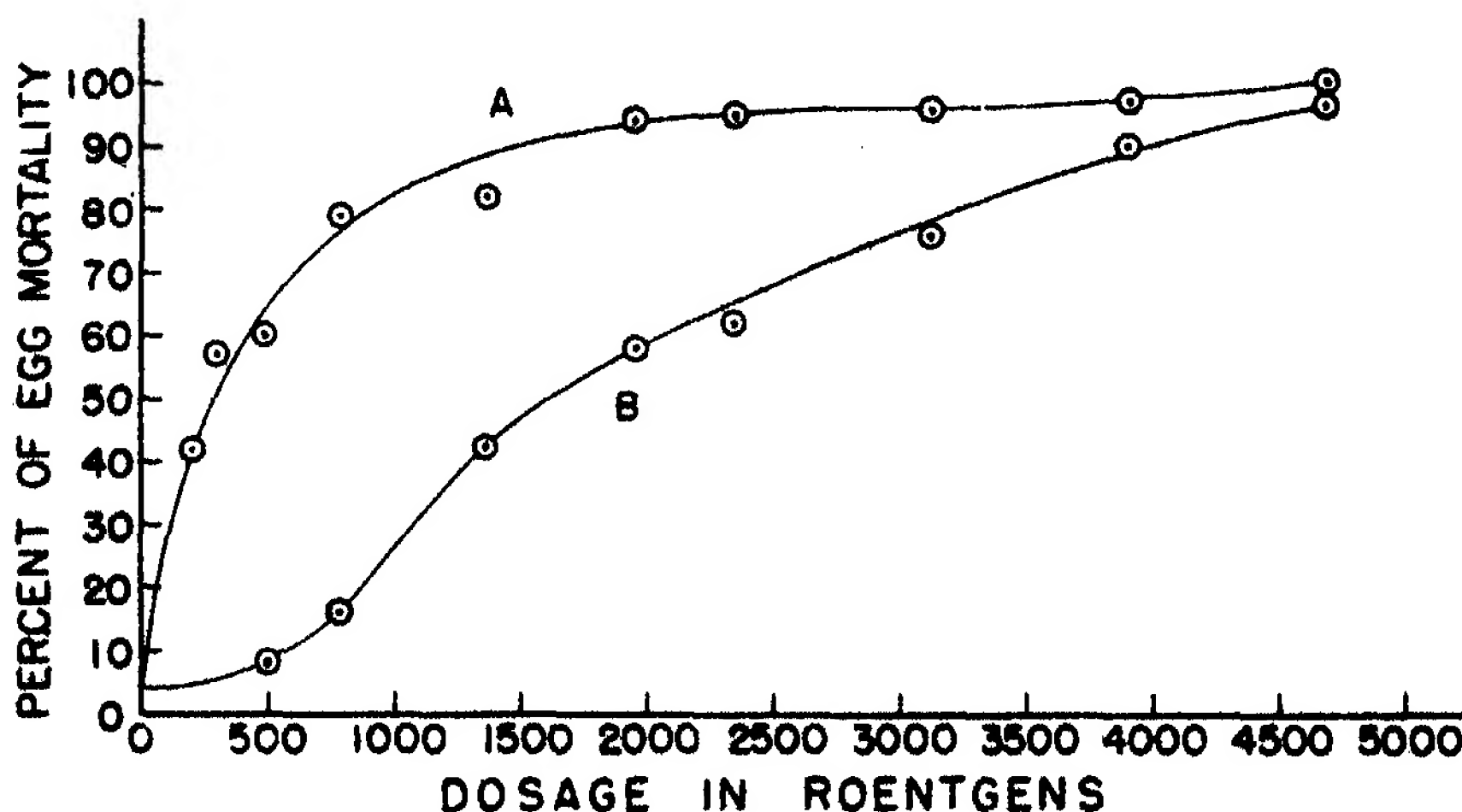


FIGURE 1

Relation between dosage and the percentage of egg mortality. (A) Adult females irradiated. (B) Adult males irradiated.

ception of the one egg which may be present in the uterus, the other components of the ovarian strings are in a stage not further advanced than metaphase of the initial meiotic division.<sup>11</sup> Following exposure, therefore, the oöcytes and oögonia have yet to pass through the two maturation divisions. Since almost all of the eggs used in this study were collected during the twenty-four-hour period after treatment (a few were collected on the second day), such eggs were the most mature, i.e., closest to first meiotic metaphase, when exposed to the radiations.

Eggs were collected as described above and counts were made in order to determine the number which eventually hatched as larvae. The data are given in table 1, while in figure 1 the percentage of egg mortality is shown plotted against dosage in roentgens. The curves suggested by the



FIGURES 2-16

2, 3. Normal metaphase and telophase of first and second meiotic divisions.  
4, 5, 6. Aberrant meiotic figures in eggs deposited by treated females.

points are non-linear and indicate the increase in egg mortality with increasing dosage. The *Y* intercept is at 3.9, representing the average egg mortality in the controls.

There is a decided difference in the sensitivity of the sperm and mature oöcytes to the radiations. The effects of small dosages on the female gametes are very striking. This is evident in the graph, for with dosages up to 780 r the curve *A* rises steeply; from this point the slope diminishes and the curve gradually approaches the maximum. These data follow closely those of A. R. Whiting<sup>12</sup> who irradiated *Habrobracon* females and determined from daily egg counts that mortality was highest for eggs laid on the first day following the treatment. Curve *B*, representing the variations in egg mortality following exposure of the sperm, has a gradual slope and only at the highest dosage tends to approximate the other curve. The number of affected male gametes, while not directly proportional to dosage, increases gradually.

TABLE 1

DOSAGE IN ROENTGENS	ADULT FEMALES IRRADIATED (X UNTREATED MALES)			ADULT MALES IRRADIATED (X UNTREATED FEMALES)		
	EGGS DEPOSITED	EGGS NOT HATCHING	% EGGS NOT HATCHING	EGGS DEPOSITED	EGGS NOT HATCHING	% EGGS NOT HATCHING
195	458	192	42.1	....	...	....
293	752	432	57.4	....	...	....
488	1080	656	60.7	444	36	8.1
780	564	448	79.4	296	48	16.2
1365	538	454	82.2	498	212	42.5
1950	568	536	94.6	620	360	58.1
2340	772	734	95.1	740	460	62.1
3120	552	528	95.7	1224	928	75.8
3900	530	516	97.4	594	528	88.9
4680	144	144	100.0	251	242	96.5

(b) *The Maturation Divisions*.—Entrance of the sperm normally induces the completion of the first meiotic spindle. The chromosomes on the first polar spindle advance as far as metaphase and then pause until the sperm enter the egg, whereupon the divisions proceed. The anastral maturation divisions occur close to the dorsal surface of the egg in an island of protoplasm situated approximately one-fourth of the distance back from the anterior end. The chromatic complexes which separate during the first meiotic division form no vesicular nuclei, but, after a few minutes pass directly upon the second polar spindles (Figs. 2 and 3). The second division proceeds, resulting in four haploid nuclei of which the innermost becomes the female pronucleus. The other three are the polar body

FIGURES 2-16 (Continued)

7-16. Types of cleavage figures observed in eggs with treated chromosomes. Fragmentation, chromosome clumping, multipolar configurations are commonly seen. Similar aberrations are noted whether sperm or egg contributes the irradiated chromosomes.



nuclei; these are not extruded from the egg, but remain in the antero-dorsal island and there gradually disintegrate.

In the sections of eggs laid by treated females and fixed between five and sixteen minutes after deposition, various distortions of the maturation figures can be observed. No achromatic figure (Fig. 4) may be present, while the fractured chromosomes are scattered about in the clear protoplasmic island. That this egg was in the stage of polar body formation is indicated by the presence of an unresolved sperm head with its centriole and delicate sperm tail in another section. Occasionally a part of the spindle is missing. The remainder may not have formed at all or, having formed, disintegrated earlier. For example, in figure 5 only a half-spindle with a chromosomal mass at the remaining pole is present; several chromatic elements are lying free in the cytoplasm. In the treated material multipolar spindle configurations rather than the normal bipolar arrangements are sometimes present (Fig. 6). No sperm tails were seen in the island in which the division represented by figure 6 was occurring, thus precluding the possibility that a supernumerary spermatozoon may have established an independent spindle which combined with the first polar body spindle to upset the process of meiosis. The chromosomes and chromosome fragments are distributed over all three poles and it is difficult to establish whether the complex at any one pole is a balanced one. While the maturation divisions are occurring, one of the sperm heads normally becomes large and vesicular and eventually fuses with the resulting female pronucleus. However, if as a result of aberrant divisions no female pronucleus is formed, the sperm head may resolve itself into its haploid complement of chromosomes, but no instance was ever observed in which a haploid sperm complement formed a mitotic figure and began to cleave. In one hundred preparations of early normal eggs, selected at random, only one aberrant meiotic figure was noted. Within the sections of sixty-nine organisms whose mothers had been treated with 2340 r, there were, on the other hand, sixteen with various meiotic abnormalities.

(c) *The Early Cleavages.*—During the early cleavages, the egg is a syncytium with the nuclei uniformly scattered throughout the cytoplasm. The nuclei divide synchronously and, at the eighth cleavage, begin their migration to the periphery, arriving in the posterior polar plasm slightly prior to their appearance in the other portions of the egg. Globules of posterior polar plasm, each containing a nucleus and a number of the polar granules, are pinched off; these are the primordial germ cells.

In these normal eggs the mitotic figures are bipolar and equivalent chromosomal groups separate at anaphase. This orderly mode of mitosis is frequently found upset, however, in zygotes developing with treated chromosomes.<sup>18</sup> In such zygotes, chromosome fragmentation, chromatin bridges, asymmetrical divisions, clumping of the chromosomes, multipolar

figures of the tripolar and quadripolar types and cytoplasmic vacuolization have been observed. Within the sections of a single egg, there may be found chromatic and achromatic aberrations of various kinds, as well as apparently normal figures. Furthermore, similar aberrations appear in the eggs, regardless of whether sperm or ovum contributed the irradiated chromosomes. It would be impossible to indicate all of the abnormalities noted in the zygotes and the varied modes of interference with the progress of normal development. Therefore, a representative group has been selected for discussion.

A first cleavage figure, with no evidence of gonomery, is indicated in figure 7. Fracture of one or more chromosomes had occurred, and elimination of some fragments appears to be taking place, since some are off the spindle and lying free in the cytoplasm. Other fragments are passing to the poles and may be incorporated in a daughter nucleus. In a slightly later developmental stage, late prophase of the second division, the nuclear membranes are beginning to disappear but the chromosomes are distinct (Fig. 8). An asymmetrical distribution had evidently occurred during the progress of the first division, for the volume of chromatin present in one of the nuclei is definitely greater than in the other nucleus. Lying close to the lower figure are some fragments which stained lightly and which had not been included in the reconstruction nucleus.

Irregularity in the mechanics of mitosis is well manifested through the frequently observed multipolar figures which are found in the sections of eggs of treated parents. Several of these atypical spindle arrangements may be observed within one egg. The chromosomes and fragments are usually found distributed over the various spindles (Figs. 9, 10 and 11), but are also observed clumped at one of the poles (Fig. 12). With the exception of a rod-like fragment, all of the chromosomes in the latter figure are massed at one pole, forming an irregularly shaped clump which stained intensely. Clumping of the chromosomes is often noticed in the experimental material. The radiations alter the consistency of the chromosomes and the change is usually manifested at anaphase when the viscid strands are about to separate. The pycnotic masses take various shapes (Figs. 12, 13, 14, 15 and 16). At times the clumped masses may be connected by chromosomal strands, but often they appear as more or less solid complexes in which the identity of the individual components is lost. It has not been possible to determine whether the complexes at the poles in figures such as have just been referred to can continue to divide. If restitution nuclei do form and cleavage does continue, nuclei with varying chromosome combinations would result.

Indication has already been given above that irregularities in the mitotic mechanism are by no means restricted to one particular type in any one organism. A tabulated presentation of the occurrence of such

abnormalities in five organisms which were fixed during the early cleavage divisions is given in table 2. It is interesting to note that among the degenerate aberrant figures lie some which are apparently normal and appear to be capable of continuing to divide.

TABLE 2

SEX OF TREATED PARENT	DOSE	NUMBER AND TYPE OF MITOTIC ABNORMALITIES
Male	3900 r	11 figures containing clumped chromatic masses; 1 quadripolar figure
Male	3900 r	3 figures with fractured chromosomes; 1 normal figure
Male	3120 r	1 tripolar figure; 3 figures with clumped masses; 1 normal figure
Female	2340 r	3 figures with chromosomes and fragments
Female	2340 r	2 figures with chromosomes and fragments; 1 normal figure

(d) *Later Embryonic Stages.*—Development and morphogenesis proceed at such a rate in *Drosophila* that after twelve hours of incubation at 25°C. the egg has passed from first cleavage to a relatively highly complex, differentiated organism. During this period, the syncytial cleavages occur, the primordial germ cells are segregated, the malpighian tubules and nervous tissue have made their appearance, metamerism has occurred, the definitive number of salivary gland cells is present, the major portion of the yolk has been enclosed in the mid-gut and a pair of gonads are in their permanent position in the postero-dorsal region of the embryo. Later, other structures appear and during the twentieth and twenty-first hour of development a majority of the young larvae emerge from the embryonic cases. Among the offspring of treated adults are found organisms which apparently differ in no morphological aspect from controls of similar age, together with embryos in which extensive cellular proliferation but no differentiation has occurred. Although the latter are from twelve to sixteen hours of age, no sign of structural organization is noticed. Apart from the yolk inclusions which are concentrated in one or another region of the organisms, these embryos consist of massed aggregates of cells. The cells are of various sizes, often vacuolated and their membranes are frequently not intact, resulting in syncytial-like patches. The cells have no orderly arrangement, but are haphazardly dispersed throughout the cytoplasm. No embryo comparable to these was observed in the controls.

Eggs which had not hatched by the third day following deposition, i.e., some forty-eight hours past the normal modal hatching time, were selected at random and dechorionated. In some of the dechorionated eggs no evidence of development could be seen, but in others embryonic differentiations were visible. These embryos were segmented and larval jaws and tracheae were noted. When removed from the membranes, such embryos would sometimes move their jaws and muscles feebly; others remained in the position in which they were placed and showed no activity at all. In



several instances, the larvae had difficulty in emerging wholly from the egg cases, for the envelopes still enclosed the posterior portion of these organisms. Careful removal of the membranes did not induce such individuals to move or show any other activity.

*Summary.*—When *Drosophila* males and females are exposed to x-rays and then mated with untreated flies, a high percentage of the embryos do not hatch as larvae. Especially susceptible to the radiations are the most mature oöcytes. The treatments have affected the gametes to an extent that, in many cases, orderly development becomes impossible. Varied distortions of the meiotic and mitotic divisions have been observed. Within the sections of single eggs, distortions of various types as well as apparently normal figures have been found. At an age when normal embryos are highly differentiated, some of the embryonic offspring of irradiated parents consist of structureless masses of cells. It is likely that there is a direct relationship between the observed cytologic and developmental abnormalities and the greatly increased percentage of mortality among the progeny of treated adult *Drosophila*.

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<sup>10</sup> Huettnner, A. F., *Zeit. Zellforsch. und mikrosk. Anat.*, **11**, 615-637 (1930).

<sup>11</sup> Huettnner, A. F., *Jour. Morph. and Physiol.*, **39**, 249-265 (1924).

<sup>12</sup> Whiting, A. R., *Jour. Exp. Zool.*, **83**, 249-269 (1940).

<sup>13</sup> Bauer, H., Demerec, M., and Kaufmann, B. P., *Genetics*, **23**, 610-630 (1938).

These investigators have analyzed the salivary gland chromosomes of first generation larvae, obtained by mating irradiated males with untreated females, for chromosome alterations induced in the treated sperm.

ON THE LAW FOR MINIMAL DISCRIMINATION OF  
INTENSITIES. IV.  $\Delta I$  AS A FUNCTION OF INTENSITY

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Communicated May 7, 1940

The nature of the functional dependence of the sensorially least discriminable increment of intensity ( $\Delta I$ ) upon the magnitude of the prevailing intensity level ( $I_1$ ) has long been recognized as presenting a problem of fundamental interest. The literature concerning it, experimental and theoretical, is full of tantalizing curiosities, and is voluminous to the point of generating stupefaction. Although enormous labor, in the aggregate, has been directed toward establishing a rational basis for the data of differential sensitivity, no even approximately satisfactory solution has been proposed thus far. The chief reason for this is to be found, I suspect, in the curious fascination of the "Weber fraction"  $\Delta I/I$ . The intensity of Fechner's realization of the psychophysical significance of the properties of  $\Delta I$  largely succeeded in fastening attention on the seductive possibilities supposed to follow upon the fact that the "Weber fraction" might be (or "ought" to be) a constant. The chief consequence of this was that the academic and essentially artificial problem of the constancy or inconstancy of the ratio  $\Delta I/I$  came to dominate a very considerable area of psychophysics.

A certain degree of sanity was introduced into this matter by Hecht's insistence<sup>1</sup> that the more realistic problem, and the theoretical opportunity, is found not in the "constancy" of  $\Delta I/I$  but in the reasons for its systematic behavior as a function of intensity. The rational theory of intensive discrimination has continued to be very largely occupied with the consideration of the properties of  $\Delta I/I^{2,3}$  or sometimes of  $I/\Delta I$ .

The reasons leading to a rejection of these considerations are of two general sorts. I am referring to the rejection of what I have just termed the current rational theory of intensive discrimination; I am not discussing those attempted formulations of the data which depend only on the use of more or less convenient but not experimentally supported equations, because any number of such formulations can be found and consequently no one of them has of itself any real analytical significance; moreover, none of them thus far detected actually describes the data or takes adequate account of their known properties. The current rational theory of intensive discrimination has had a considerable (although not a completely inclusive) success in giving descriptive formulae for the dependence of visual  $\Delta I/I$  upon  $I$ . The data of visual intensive discrimination cover a wider range of intensities, in more different kinds of organisms, than are

available with other types of sensory excitation; and they are in some important respects less complicated by the mechanical conditions of sensory reception. For reasons which Hecht has discussed in detail<sup>4</sup> it has been attractive to suppose<sup>3</sup> that the quantity  $\Delta I$  is (visually) one which produces a certain amount or rate of decomposition of photosensory receptor substance in a system adapted to  $I_1$ . The main reasons for rejecting this approach are (1) that there is no necessity for assuming that the magnitude or the properties of  $\Delta I$  are determined peripherally in the receptor,<sup>5</sup> and

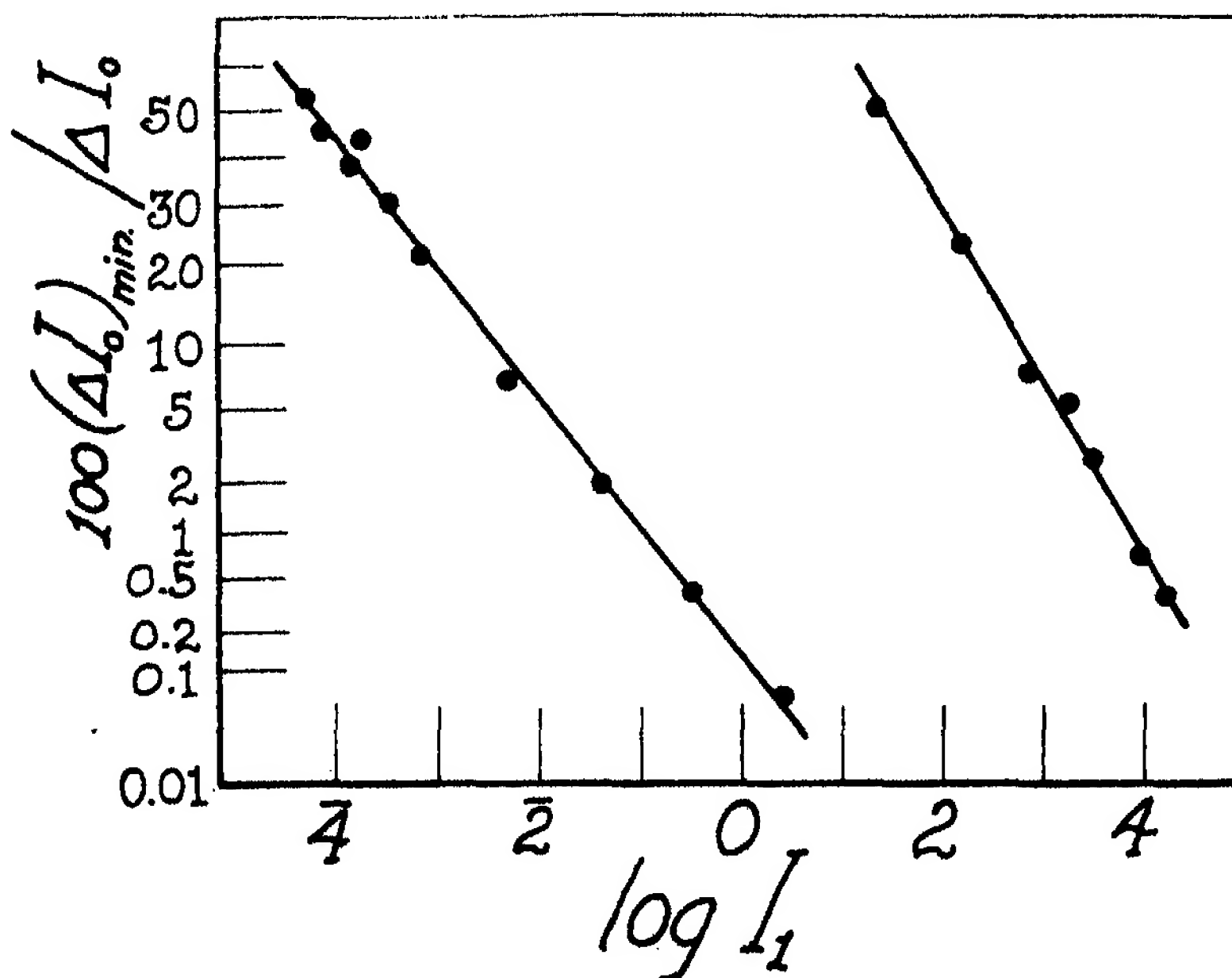


FIGURE 1

"Instantaneous" thresholds ( $\Delta I_0$ ) under conditions of adaptation to intensities  $I_1$ : "white" light data from Blanchard;<sup>13</sup> 5° field. At the left, "rod" curve, asymptotic minimum  $\Delta I_0 = 0.00589$ ; at the right, "cone" curve, minimum  $\Delta I_0 = 0.100$ . The ordinates give  $1/\Delta I_0$  as percentage of the maximum value of this fraction, on a probability grid.

(2) there is no reason whatever to accept the assumption that the threshold effect is a physically constant effect.<sup>6</sup> While the resulting equations provide a certain (but incomplete) description of the data, this is surely no argument for their uniqueness and does not by itself validate the assumptions from which they are derived.<sup>7</sup>

The matter may be approached in a quite different way, with the help of simple assumptions for which there is now considerable direct support of a totally different sort. These assumptions avoid most, if not indeed

all, of the general difficulties already referred to. The chief assumptions are: (1) the relation of sensory effect  $E$  to  $\log I$  is given by a normal probability integral; the various aspects of "effect produced" as a function of intensity, when objectively measurable, do adhere to this formulation,<sup>7,8</sup> and the reason for it is deducible in an elementary way;<sup>6</sup> (2) the reciprocal of the exciting intensity measures the capacity to be excited under the

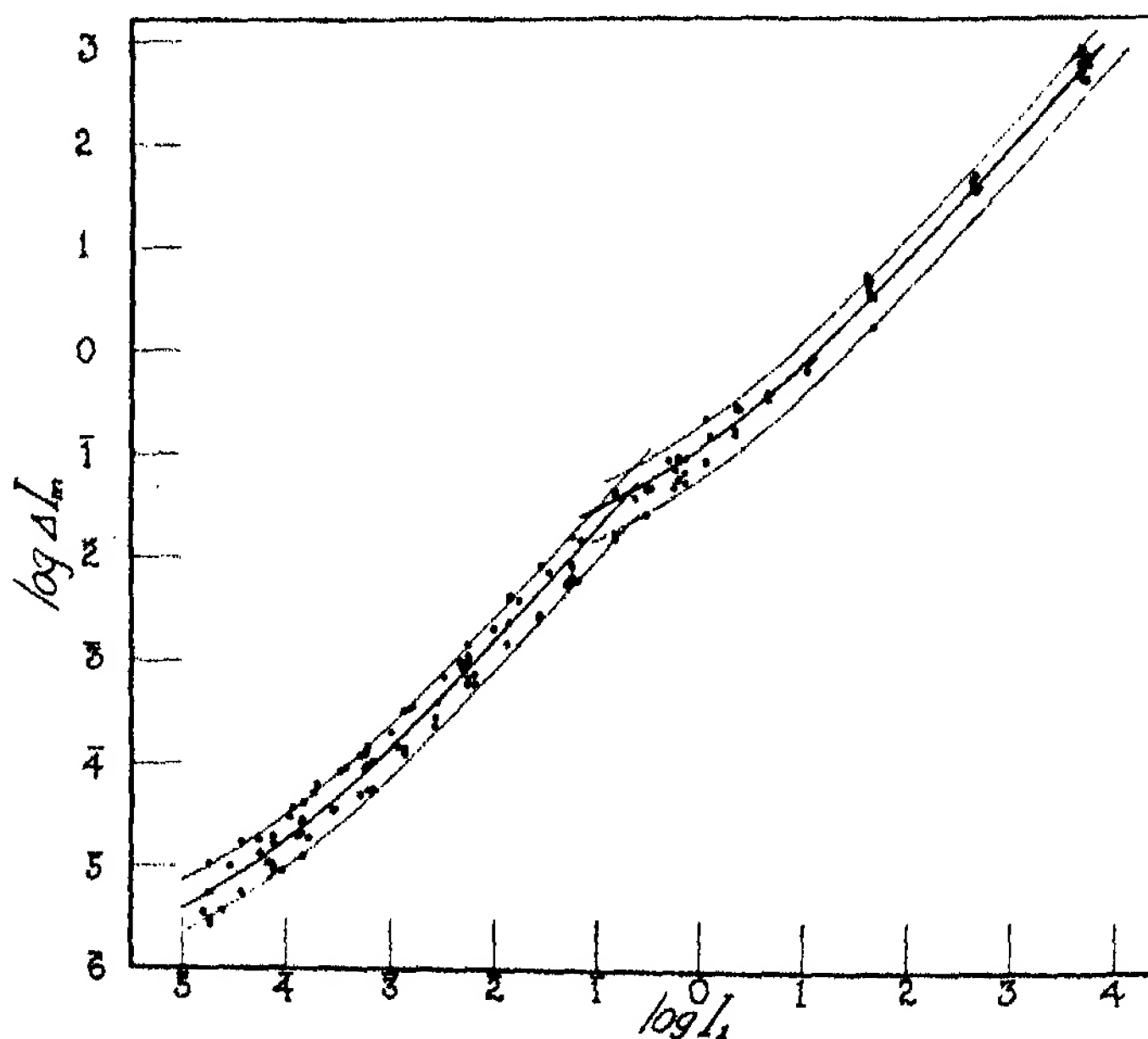


FIGURE 2

Data (cf. <sup>18</sup>) from experiments (Crozier and Holway) by the method of increasing  $I_1$  to  $I_2$ , where  $I_2 - I_1 = \Delta I$ . Each point is the mean of five measurements (left eye, W. J. C., white light, field  $30^\circ$  square centrally fixated; for observational conditions cf. <sup>5</sup>). The central lines are probability integrals for  $1/\Delta I_m$  as a function of  $\log I_1$ . The statistically constant vertical width of the band corresponds to the fact that  $\sigma_{\Delta I}$  and  $\Delta I_m$  are in direct proportion.<sup>10</sup> For the lower ("rod") segment, asymptotic minimum  $\log \Delta I_m = \bar{5}.53$ ; for the upper segment, minimum  $\log \Delta I_m = \bar{3}.80$ .

conditions and with reference to the end-point of effect employed;<sup>8,9</sup> the types of justification for this assumption are numerous, and include especially the rational behavior of the three parameters of the probability summation involving  $1/I$ , under various experimental treatments.<sup>9</sup> Viewed in this way the derivative curve of  $dE/d \log I$  as a function of  $\log I$  is of course a frequency distribution of elements of neural effects (and not of the intensity thresholds of excitable neural units).

If an assemblage of neural units is adapted to an intensity  $I_1$  the corresponding mean total sensory effect is  $E_1$ . Excitability with respect to the production of any further sensory effect by increasing  $I$  must clearly depend on the range of the distribution of  $d \log I$  still open to activation, and the range of additional effect potentially achievable will be drawn from and will be equivalent to  $E_{max} - E_1$ .

The increment of intensity producing a noticeable effect beyond that due to  $I_1$  is  $\Delta I$ . It corresponds in quantitative properties to the "absolute threshold"  $\Delta I_0$  when  $I_1 = 0$ ; the only difference is that with  $I_1$  finite the test starts from a certain level of light adaptation and  $I_1$  is (usually) still present for comparison. Among important properties in which the essential similarity of mean values of  $\Delta I$  and  $\Delta I_0$  are manifest is the rectilinear proportionality to their indices of dispersion in repeated tests,<sup>10</sup> the homologous types of dependence on retinal area<sup>6</sup> and on exposure time,<sup>6</sup> and on the level of light adaptation.<sup>6</sup> Analysis demonstrates that  $1/\Delta I_0$ , like effect  $E$ , gives a normal probability summation as a function of exposure time.<sup>6</sup> In a similar way, the "differential excitability"  $1/\Delta I$  is regarded as determined by the summation of neural effects from that part of the total potential population not already excited by  $I_1$ . In other words, the capacity to be excited, which is measured by  $1/\Delta I$ , is determined by (and thus measurable in terms of) the remaining number of unexcited elements. If effect  $E$  is a probability integral in  $\log I$ , then  $1/\Delta I$  must be a similar probability integral with reversed slope, since at each  $I_1$  we will have  $1/\Delta I$  equivalent to  $E_{max} - E_1$ .

It will be noticed that an essential part of this argument involves simply the *number* of elements of neural effect ( $dE/d \log I$ ) and makes no reference to any fixity of the individual unit thresholds in terms of  $\log I$ ; in the deduction<sup>6</sup> of the probability summation for these cases it is explicitly presumed that the thresholds of these units fluctuate at random. As a matter of fact, the relation<sup>10</sup> between  $\Delta I$  and  $\sigma_{\Delta I}$  permits another method<sup>11</sup> of obtaining the form of the  $\Delta I$  function, which emphasizes the significance of fluctuating variation of performance in the neural units concerned in statistically determining the nature of the law for sensory effect as a function of intensity; its results are essentially the same as those about to be described.

The tests of this formulation must ultimately involve a good deal more than its ability to apparently describe the data, particularly since customary criteria of curve fitting are of very doubtful utility for such data. Valid tests must be particularly directed toward examining the properties of the parameters of the function. Among the most interesting of such tests are those theoretically possible by altering the organism's temperature. But certain other tests, although in some respects less decisive, can readily be applied. The derivation of the probability summation for

$1/\Delta I$  makes no reference to anatomical specificities of receptor or central nervous organization; it makes no appeal to the photochemical (or perhaps photoelectric) basis of the primary excitation of individual visual cells; it is concerned solely with the fact that the determination of visual response is brought about through the activation of groups of neural units which fluctuate in performance and in accommodation. It should therefore be basically applicable to the data from a great variety of animals. More-

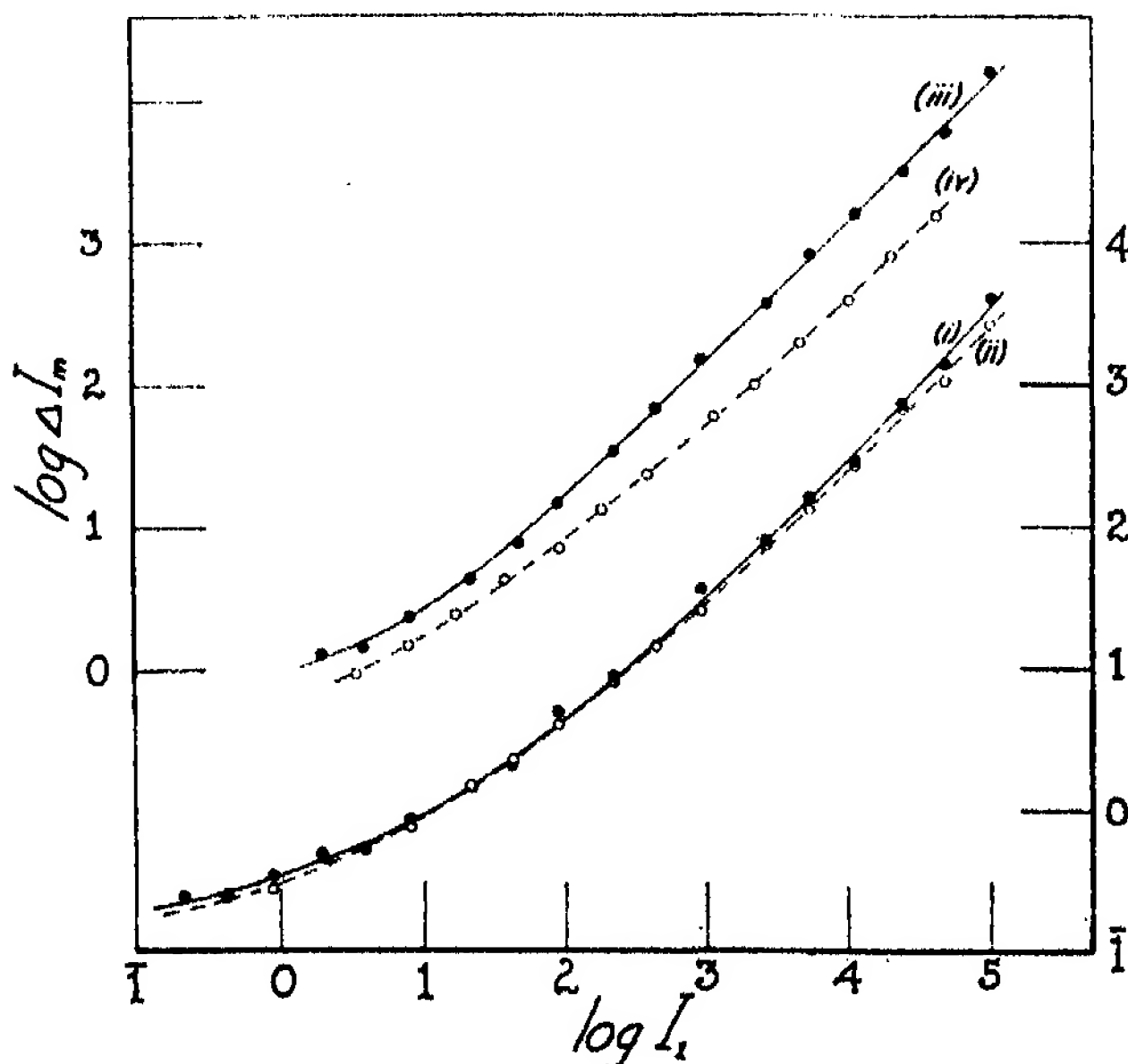


FIGURE 3

Data for small (foveal) fields, from Steinhardt:<sup>16</sup> (i), 56' test field, no surround; (ii), with an illuminated surround, the effect of the surround is to lower the theoretical minimum  $\log \Delta I_0$  from  $\bar{I}.21$  to  $\bar{I}.10$ ; (iii) and (iv), 23.5' field with and without surround (illumination relatively greater than with (ii); with (iii) the minimum  $\log (\Delta I_0)_m = \bar{I}.55$ , for (iv),  $\bar{I}.45$ . The curves are computed probability integrals for  $1/\Delta I_m$  as a function of  $\log I_1$ .

over, it should hold for "instantaneous thresholds" immediately after the removal of an adapting intensity,<sup>12</sup> for  $\Delta I$  as obtained by the method of increasing  $I$  from  $I_1$  to  $I_2$  (no contrast involved, as the illuminated field is uniform), and for the conventional  $\Delta I$  experiment in which  $I_1$  is continuously present. The experiments of these three types form a graded series as to the operational rôle of  $I_1$ , which serves to emphasize the essential nature of  $\Delta I$  as a threshold intensity under different sets of conditions.



Further, the basic rule should in principle be equally applicable for auditory and other sensory modalities. Mechanical complications due to the existence of several sets of populations of overlapping neural effects may require an extended analysis of such data. Here, only illustrative cases need be given.

Figure 1 is a plot of "instantaneous visual threshold" data from Blanchard.<sup>12</sup> These have been analyzed by Hecht<sup>13</sup> and by Federov<sup>14</sup> from the

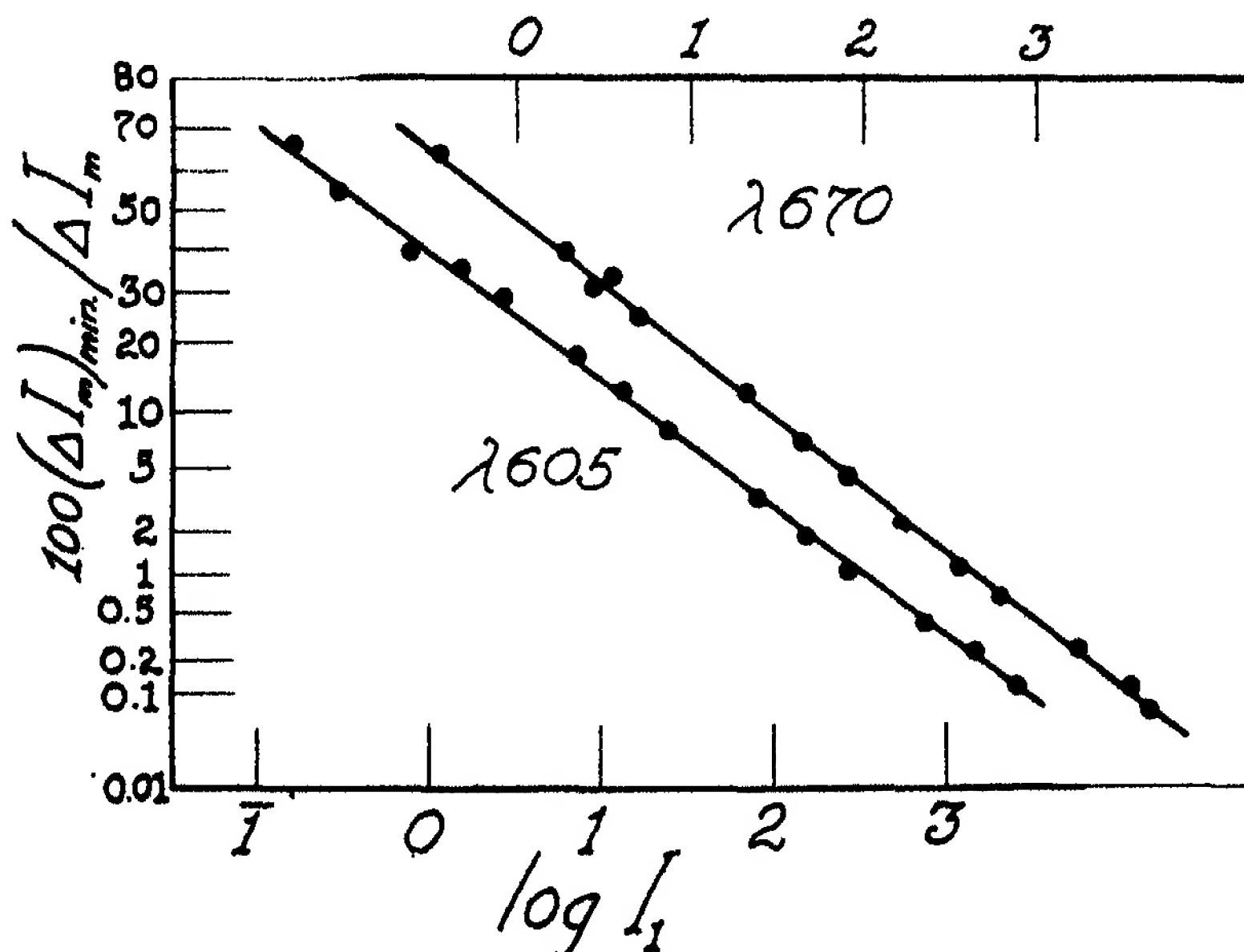


FIGURE 4

Two series of determinations (from Hecht, Peskin and Patt<sup>17</sup>) with red lights (one observer), recalculated to give  $1/\Delta I_m$  as a function of  $\log I_1$  for the "cone" segment (cf. Fig. 2, etc.), here shown on a probability grid. For  $\lambda 605$   $\log \Delta I_m = \bar{1}.52$ . For  $\lambda 670$   $\log \Delta I_m = \bar{1}.60$ .

(These data actually include several points at lower levels of  $I_1$  which "break" into the "rod" segment. It is to be remarked that when  $\Delta I/I_1$  is studied as a function of  $I_1$  the breaking of the whole range of the measurements into two segments is easily overlooked; this has sometimes occasioned the unwarranted suggestion of aberrant qualities in such data.)

photochemical standpoint, on the basis that the threshold effect is a photochemical constant. Figure 2 contains data from experiments by Crozier and Holway<sup>15</sup> using the method of increasing  $I_1$  to  $I_2$  at a constant relative rate. Figure 3 (from measurements by Steinhardt<sup>16</sup>) and figure 4 (from data by Hecht<sup>17</sup>) are from experiments involving the use of  $I_1$  and  $\bar{I}_2$  in comparison fields.

The probability integral in  $\log I_1$  efficiently describes these data, which illustrate the three main types of differential threshold measurements already mentioned. Advantages of dealing with  $\Delta I_m$  directly, rather than with  $\Delta I/I$ , are both theoretical and practical. It results that certain subsidiary questions are necessarily viewed in a new light. Thus the much discussed matter of the rise of  $\Delta I/I$  at high intensities, and the effect of a "surround" upon this, ceases to have meaning. The presence of a surround enlarges the comparison population of effects (and thus produces the expected changes in the three parameters of the  $1/\Delta I - \log I_1$  integral), but merely pushes the terminal rise of the Weber fraction to higher levels of  $I_1$  (cf.<sup>18</sup>).

*Summary.*—The differential threshold for the visual discrimination of intensities follows the same essential rules as are obeyed by the so-called "absolute" intensity threshold. When  $\Delta \bar{I}$  involves the marginal recognition of an increase of intensity above an adapted level,  $1/\Delta \bar{I}$  is a declining normal probability integral as a function of  $\log I_1$ . This is deduced on the assumption that at any given level of visual accommodation the excitability measured by  $1/\Delta \bar{I}$  is determined by the not-already-excited portion of the total population of potentially excitable neural effects. There is obtained in this way, without unnecessary assumptions, a simple, efficient formulation of the existing data which is consistent with the statistical basis of neural discriminations in general.

<sup>1</sup> Hecht, S., *Jour. Gen. Physiol.*, 7, 235 (1924–1925).

<sup>2</sup> Hecht, S., *Proc. Nat. Acad. Sci.*, 20, 644 (1934); *Jour. Gen. Physiol.*, 18, 767 (1934–1935); *Physiol. Rev.*, 17, 239 (1937).

<sup>3</sup> Steinhardt, J., *Jour. Gen. Physiol.*, 20, 185 (1936–1937); Rawdon-Smith, A. F., *Theories of Sensation*, Cambridge Univ. Press (1939), xiii + 137 pp.

<sup>4</sup> Hecht, S., *A Handbook of General Experimental Psychology* (ed. C. Murchison), Clark Univ. Press, Worcester (1934), pp. 704–828; *Physiol. Rev.*, 17, 239 (1937).

<sup>5</sup> Crozier, W. J., and Holway, A. H., *Jour. Gen. Physiol.*, 22, 341, 351 (1938–1939a, b); *Ibid.*, 23, 101 (1939–1940); Crozier, W. J., *Proc. Nat. Acad. Sci.*, 26, 54 (1940a); *Ibid.*, 26 (in press) (1940b).

<sup>6</sup> Crozier, W. J., *Ibid.*, 22, 412 (1936); *Ibid.*, 26, 54 (1940a); *Ibid.*, 26 (in press) (1940b).

<sup>7</sup> Crozier, W. J., *Ibid.*, 23, 71 (1937); Crozier, W. J., Wolf, E., and Zerrahn-Wolf, G., *Jour. Gen. Physiol.*, 21, 17 (1937–1938a); *Ibid.*, 21, 223, 313 (1937–1938b, c); *Ibid.*, 22, 311, 451 (1938–1939a, b); Crozier, W. J., and Wolf, E., *Ibid.*, 22, 795 (1938–1939); *Proc. Nat. Acad. Sci.*, 25, 171, etc. (1939).

<sup>8</sup> Crozier, W. J., *Ibid.*, 25, 78 (1939); Crozier, W. J., and Wolf, E., *Ibid.*, 25, 171 (1939); *Jour. Gen. Physiol.*, 23, 143 (1939–1940a, b); 23 (in press).

<sup>9</sup> Cf.<sup>1,2</sup>.

<sup>10</sup> Crozier, W. J., *Ibid.*, 19, 503 (1935–1936); *Proc. Nat. Acad. Sci.*, 22, 412 (1936); Holway, A. H., and Crozier, W. J., *Ibid.*, 23, 509 (1937); Crozier, W. J., and Holway, A. H., *Ibid.*, 24, 130 (1938); *Jour. Gen. Physiol.*, 23, 101 (1939–1940).

<sup>11</sup> Discussed in the following paper of this series.

<sup>12</sup> Blanchard, J., *Physiol. Rev.* (Ser. 2), 11, 81 (1918).

- <sup>13</sup> Hecht, S., *Proc. Nat. Acad. Sci.*, 23, 227 (1937).  
<sup>14</sup> Federov, N. T., *C. R. Acad. Sci. (URSS)*, 24, 696 (1939).  
<sup>15</sup> Crozier, W. J., and Holway, A. H., to be described elsewhere.  
<sup>16</sup> Steinhardt, J., *Jour. Gen. Physiol.*, 20, 185 (1936-1937).  
<sup>17</sup> Hecht, S., Peskin, J. C., and Patt, M., *Ibid.*, 22, 7 (1938-1939).  
<sup>18</sup> Crozier, W. J., *Proc. Nat. Acad. Sci.*, 22, 412 (1936); a fuller account of this matter will be published elsewhere.
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## DIFFERENCES BETWEEN MEN AND WOMEN IN THEIR RESPONSE TO HEAT AND COLD

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Read before the Academy, April 22, 1940

There have been a large number of publications dealing with the reactions of men to changes of environmental temperature, but very few studies with women. Just why there has been such a neglect of half our population is not clear. Air conditioning is theoretically based on physiological studies, and politeness alone would demand more consideration of the ladies. It is well known that women in light clothing tolerate a wide range in temperature. This is not due to greater fortitude or vanity but rather a better physiological adjustment.

Max Rubner in 1890 and 1902<sup>1-3</sup> outlined the factors concerned in the loss of heat and described the "physical regulation" through changes in heat loss or voluntary activity. He also spoke of a rise in heat production through "chemical regulation" which is induced without visible changes in the activity of the animal. In previous papers from the Russell Sage Institute of Pathology<sup>4,5</sup> presented before this Society we reported that the heat production of the two normal men studied by us in detail was uniform when exposed to temperatures between 22° and 35°C. This is in accordance with the findings of Wiley and Newburgh<sup>6</sup> in Ann Arbor, Winslow, Herrington and Gagge<sup>7</sup> in New Haven, and others. Martin,<sup>8</sup> however, did note a falling metabolism in one man during a voyage to the tropics. There have been many reports indicating that the basal metabolism of men and women is lower in the tropics, but there are several factors involved besides temperature. Miss Mason,<sup>9</sup> who eliminated all the factors except climate, observed that the metabolism of a group of women was 5% lower in tropical India than it was in the temperate zone. Hardy and Milhorat,<sup>10</sup> in a brief preliminary publication from the Russell Sage Institute of Pathology last year, demonstrated that three normal women showed a considerable drop in metabolism in a warm environment and

they analyzed the differences between these women and the two men that we had studied in the same manner. In this present report we can add the results on four more women.

The men and women were studied in the respiration calorimeter of the Russell Sage Institute of Pathology<sup>11</sup> which is now at the New York Hospital. They came to the laboratory at 9 o'clock in the morning without breakfast, sat quietly for an hour in the calorimeter room which had been kept at the desired temperature since the previous day, undressed and entered the calorimeter which was then sealed. During the preliminary period in the box the surface temperature was measured. The first experimental period was started at about 11 o'clock, and observations were terminated either at noon or at 1 o'clock. At the end of the experiment the surface temperature was determined once more. During the experimental periods the subjects lay almost motionless on a comfortable bed made of fish net with a folded sheet under the body. There was little movement of air in the calorimeter. The two normal men were 33 and 54 years old, in good physical condition; and the seven women were artist models or technicians, in good physical condition, between the ages of 21 and 35 years, with weights of 54 kg. to 77 kg. They were all intelligent and coöperative.

The respiration calorimeter of the Russell Sage Institute of Pathology has been described many times. Since 1935 we have been using the Hardy radiometer<sup>12</sup> to determine skin temperature. Combining these two instruments the following quantities are measured:

- (1) The rectal temperature, measured every 4 minutes
- (2) The skin temperature in twenty points, at the beginning and end of each period
- (3) The calorimeter temperature (wall and air), at the beginning and end of each period
- (4) The total heat production during a period (usually one hour)
- (5) The total heat loss by radiation and convection
- (6) The total grams of water vaporized from skin and lungs
- (7) The surface area of the body

From these data, according to procedures already described in detail, the following quantities could be calculated:

- (1) The average skin temperature
- (2) The effective radiating surface of the skin
- (3) The total heat loss by radiation
- (4) The total heat loss by convection
- (5) The total heat loss by vaporization

- (6) The heat stored in the body
- (7) The conductance of the peripheral tissues of the body
- (8) The cooling constant of Newton's Law of Cooling

Figure 1, which has already been described in previous publications<sup>4,5</sup> shows the results on the two men. It will be noted that the heat production represented by the first blank column in each group of experiments

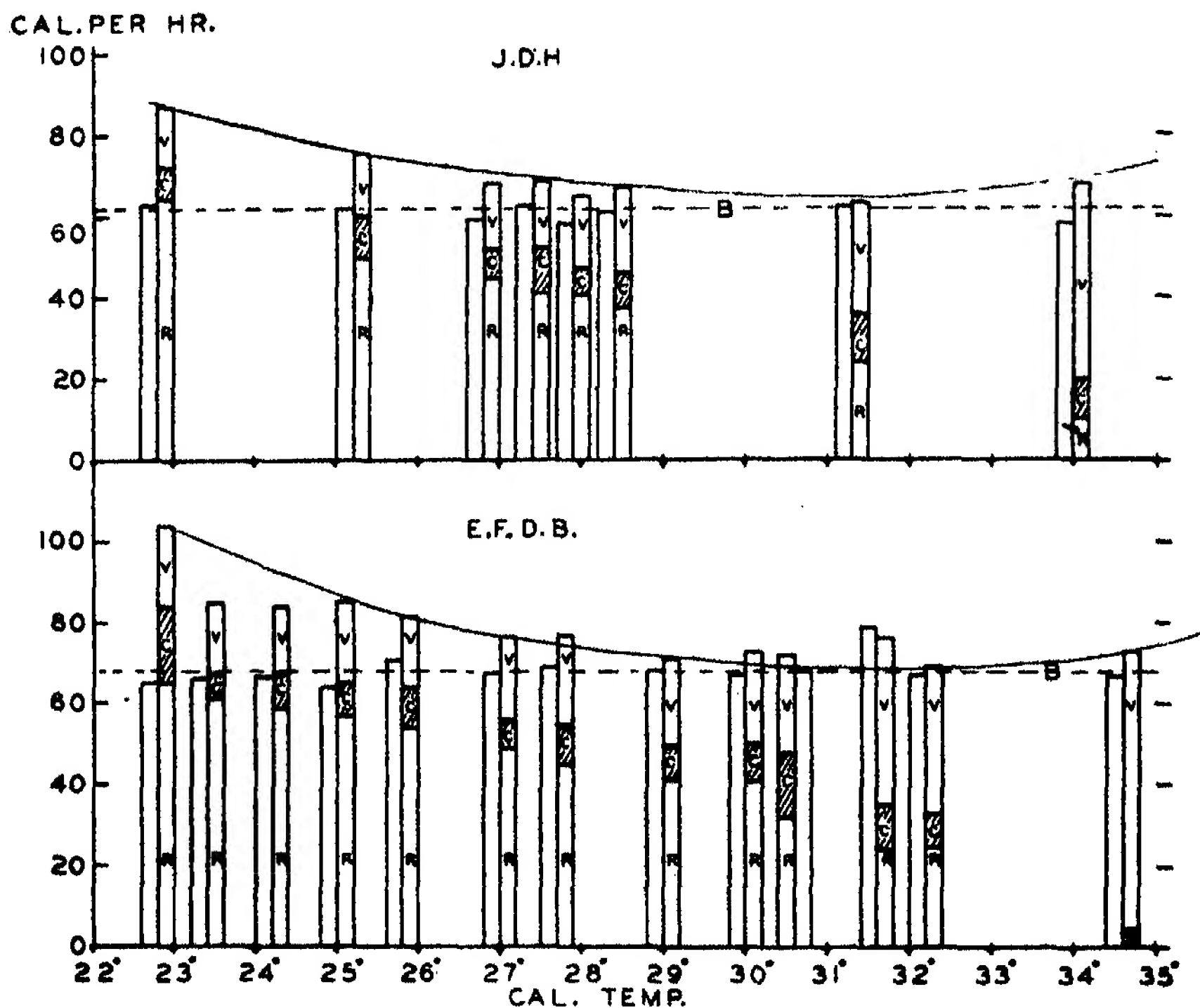


FIGURE 1

Heat production and heat loss of two normal men exposed naked in the calorimeter to temperatures between 22-35°C. The first or blank column in each experiment shows the basal heat production (*B*) which was uniform throughout the range. The second column shows heat loss divided into radiation (*R*), convection (*C*) and vaporization (*V*).

remained constant throughout the experimental range. The heat loss shown in the second column of each group exceeded the heat production in the cold zone and equalled it in the comfort zone between 29° and 31°C. Radiation (*R*), given in the lower portions of each of these heat loss columns, decreased steadily with increasing temperature until it disappeared at 35°C. when the skin temperature equalled the temperature of the wall of

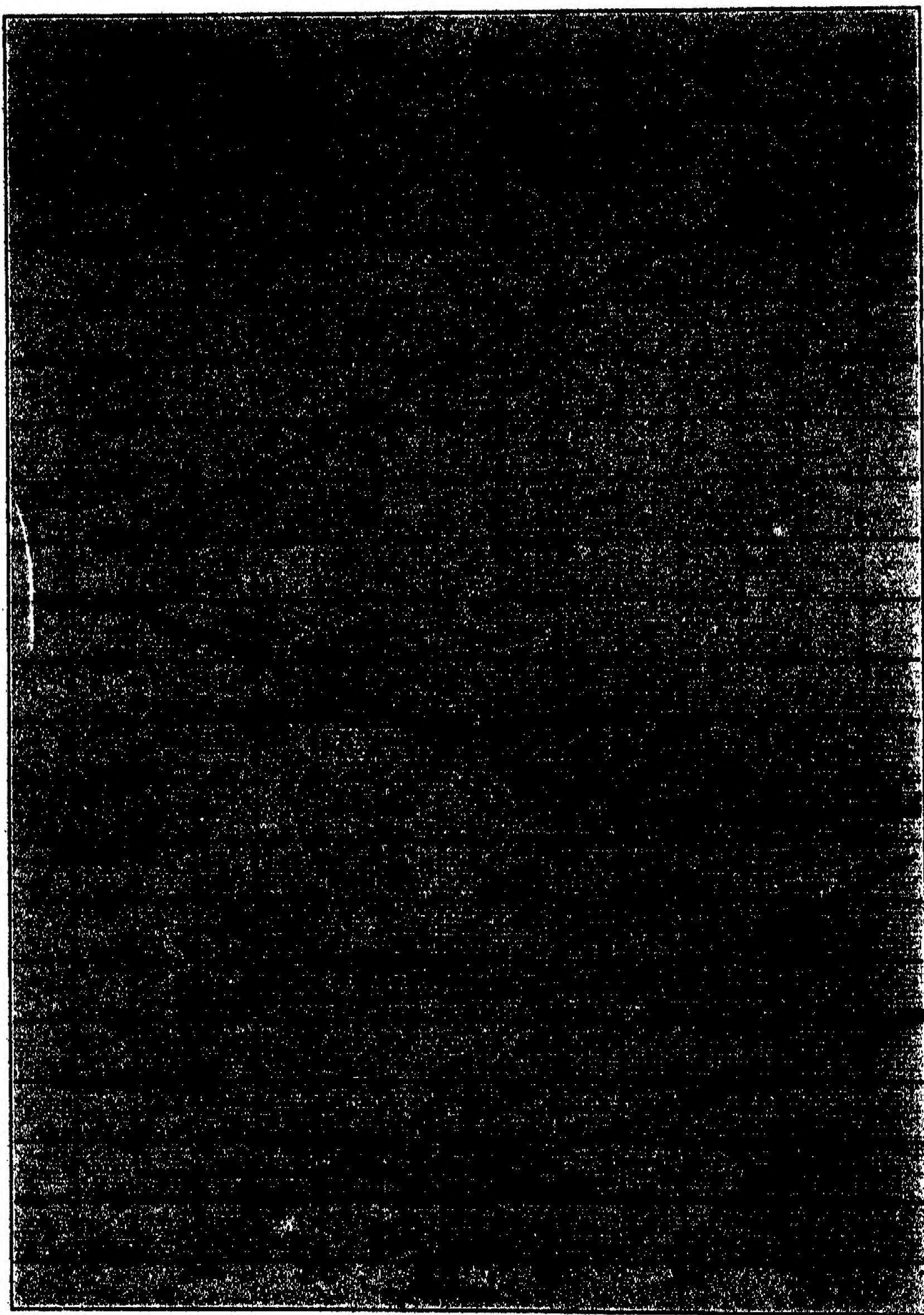


FIGURE 2

(Figures 2 and 3.) Calorimeter data obtained in two normal women showing the fall in heat production in temperatures above 27°C.

the calorimeter. Convection (*C*) played a relatively small rôle because there was slight air movement in the calorimeter and loss by convection



disappeared when the air temperature was the same as the skin temperature. Vaporization ( $V$ ) remained fairly uniform until the air temperature rose above  $30^{\circ}\text{C}$ ., then increased rapidly until it assumed the whole burden of heat loss at  $35^{\circ}\text{C}$ .

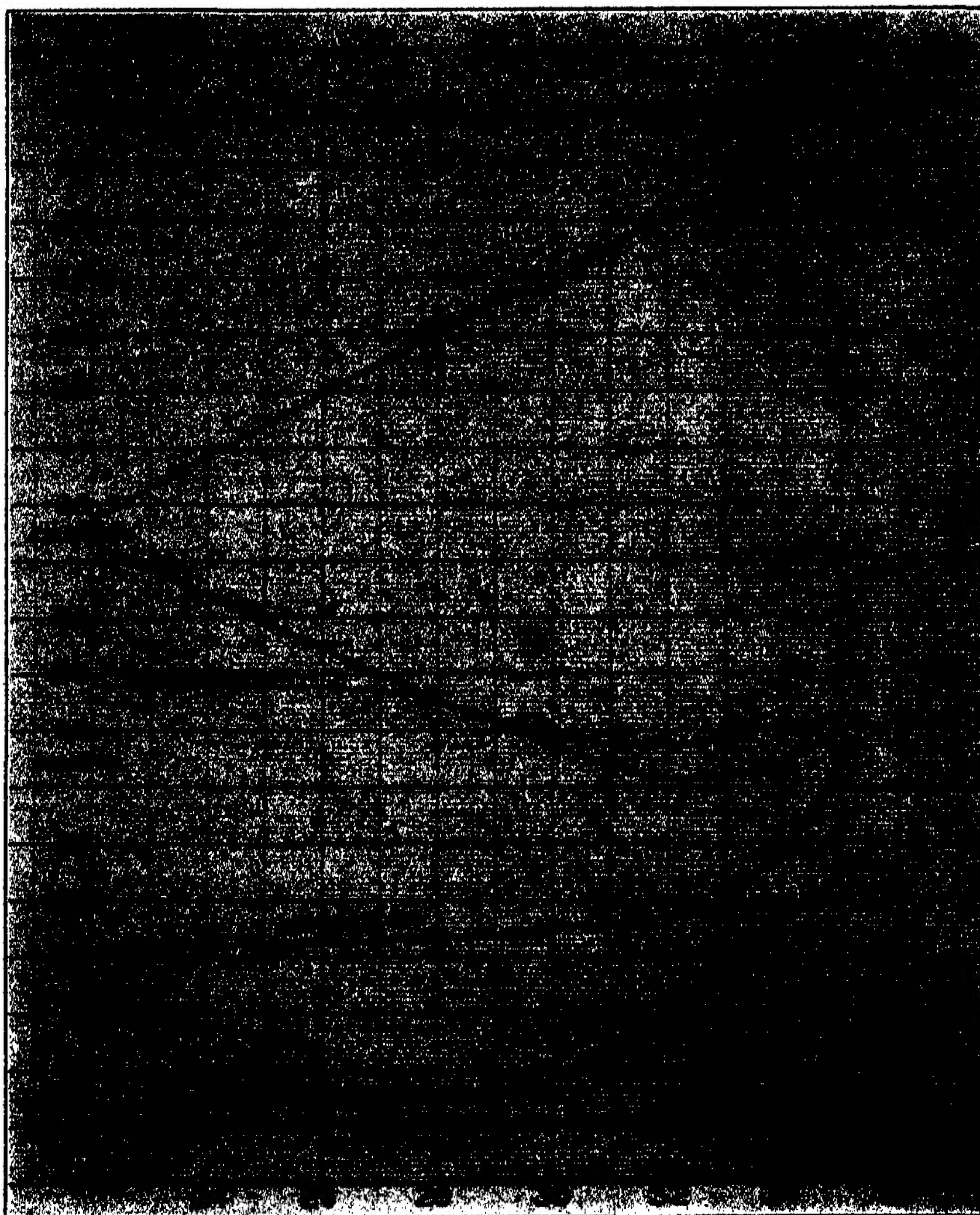


FIGURE 3

The results on three of the young women are shown in figures 2, 3 and 4. The first two demonstrate a marked fall in heat production in the warm zone. The third woman was an exception, for there was practically no

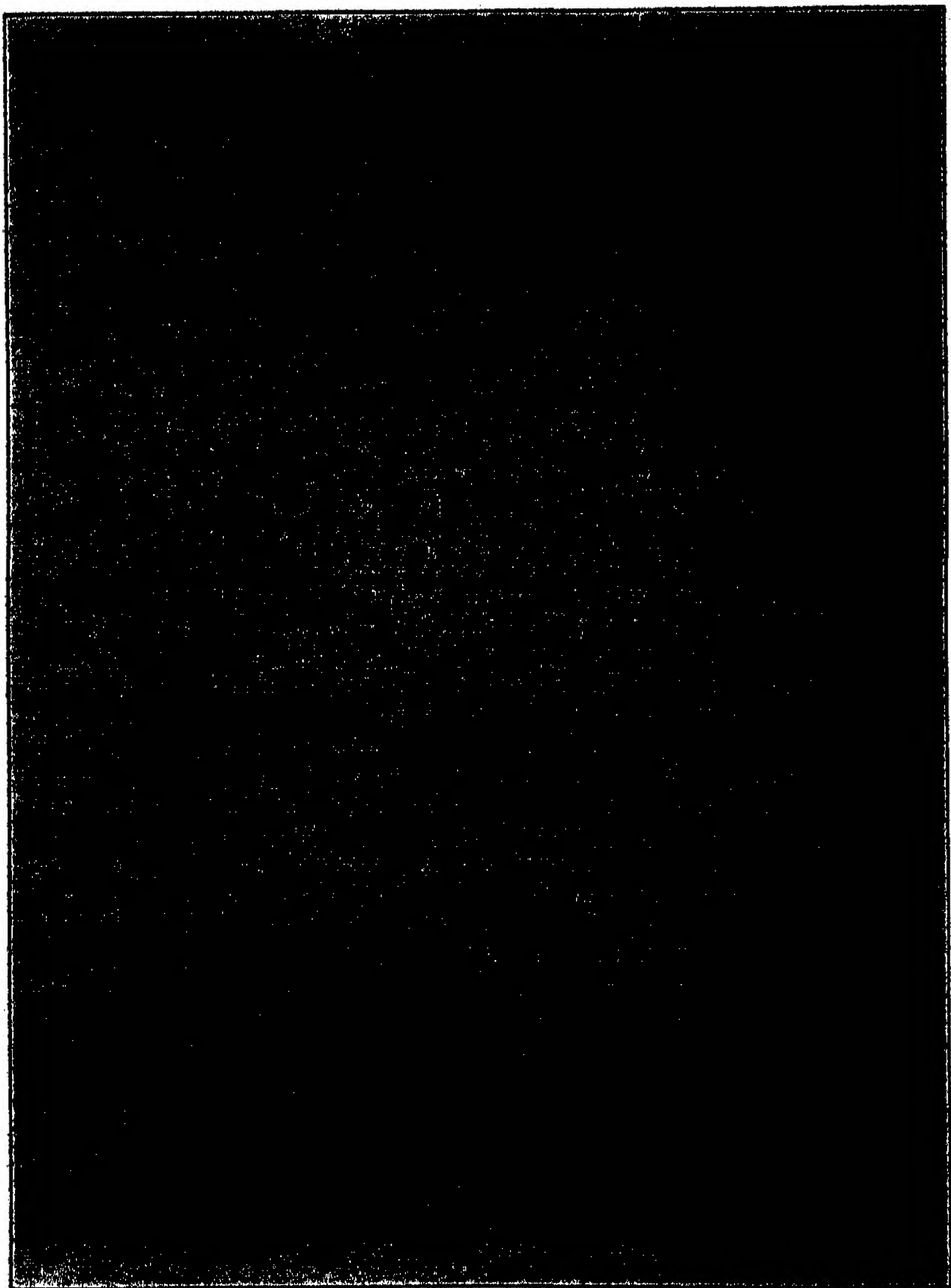


FIGURE 4

The one woman in a series of seven who showed no fall in heat production with rising temperatures.

change in heat production between air temperatures of  $24.5^{\circ}$  and  $34^{\circ}\text{C}$ . It so happens that this woman was being treated with hormones for amenorrhea. Whether or not this is significant we do not know. Figure 5

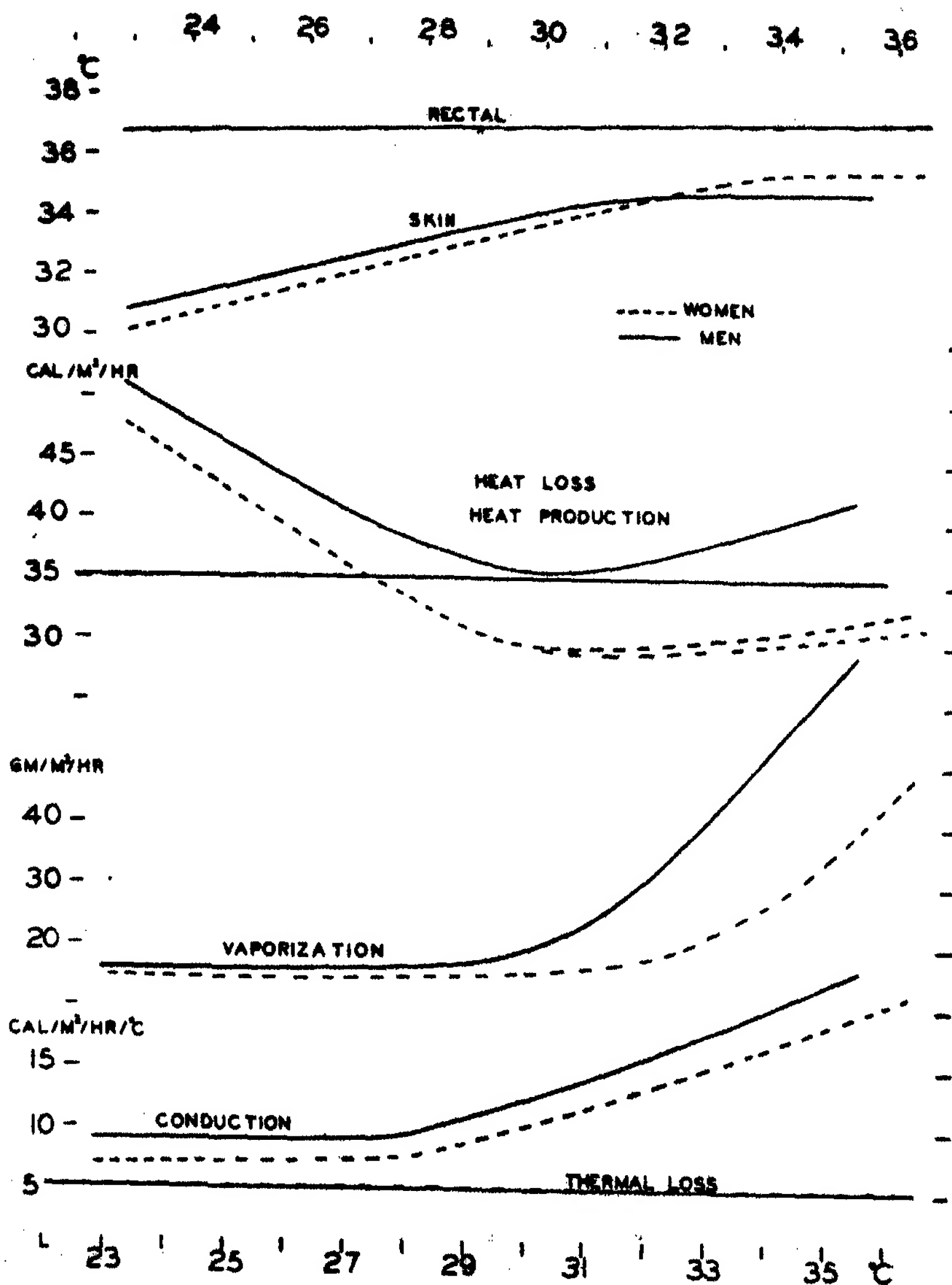


FIGURE 5

A comparison of results obtained on men and women at calorimeter temperatures between 22–35°C. Note that the dotted line showing the heat production of the women is almost exactly the same as that of the men in temperatures between 23–27°C. and then falls, almost equaling the upper dotted line which shows the heat loss in women.

shows the differences between the results obtained on the men and women. The measurements made after about 2½ hours' exposure, naked, and motionless, to the various temperatures, are plotted according to the

calorimeter temperature in degrees Centigrade. Needless to say it required many days to complete the tests on any one subject.

(1) The rectal temperatures were the same for the men and women and were both slightly lower in the cold zone as a result of exposure to the cold air. There was a spread of about  $0.6^{\circ}\text{C}$ . in the individual measurements.

(2) After exposure to the cold air for approximately  $2\frac{1}{2}$  hours the average skin temperature of women was about  $1.0^{\circ}\text{C}$ . lower than that of the men. In the warmest zone skin temperatures after the same exposure were about  $1.7^{\circ}\text{C}$ . higher than those of the men. In environments colder than  $33^{\circ}\text{C}$ . the average skin temperature fell almost linearly with the calorimeter temperature so that a change of  $1^{\circ}$  in the calorimeter caused a change of  $0.5^{\circ}$  in the skin. The change in the women was greater than that observed in the men by  $0.1^{\circ}\text{C}$ . per degree fall in the environmental temperature.

(3) The heat loss for women in the cold zone was about 10% lower than that of the men on account of the lower skin temperature. In the warm zone the heat loss of most of the women was about 14–20% lower than that of the men because the women did not sweat as much.

(4) The heat production of the men was constant throughout the temperature range. In marked contrast to this the heat production of the women which was the same as that of the men in the cold zone showed a marked fall in temperatures about  $27^{\circ}\text{C}$ . Above this point the heat production of the women fell with the heat loss being almost equal to it at all times. Therefore, in temperatures from  $30^{\circ}$  to  $32^{\circ}\text{C}$ . under our standard conditions the metabolism of most of the women was 14–20% lower than that of the men.

(5) Vaporization in the cold zone is accounted for by the loss of water from the respiratory passages and by seepage of moisture through the skin. There is no appreciable activity of the sweat glands in the cold. In this zone the vaporization was approximately equal in men and women, but as the temperatures grew warmer the men started to sweat at  $29^{\circ}\text{C}$ . The women did not start until the air temperature had been raised to about  $32$ – $33^{\circ}\text{C}$ . They did not need to sweat as soon or as much because they had lowered the production of heat in their bodies. At temperatures around  $34$ – $36^{\circ}\text{C}$ . both sexes lost all their heat through vaporization because the skin temperature had equaled the air and wall temperatures and radiation and convection had disappeared as channels of heat loss.

(6) Conductance, (conduction, conductivity) represents the ease with which heat is transferred from the interior of the body at a temperature of  $37^{\circ}\text{C}$ . to the surface, which is at a much lower temperature. Conductance can be calculated from the total heat lost in hourly periods knowing the rectal temperature and the average skin temperature. Under all conditions heat is transferred by conduction through the skin and subcu-

taneous tissues, which are about as good insulators as cork. In the cold these tissues are almost bloodless. The lower conductance for women in the cold is due to the greater thickness of the subcutaneous tissues, and we calculate that the average difference represents a layer of fat about 4 mm. thick. We have found that fat has about the same conductance as muscle, skin or cork.

The upward swing of the curves in both sexes which begins at 28°C. is due to a new factor, the steadily increasing transport of heat by the blood stream. With rising environmental temperatures there is an increased vaso-dilatation. Although the hands and feet show the most dramatic changes in blood flow they play a relatively insignificant part since they represent only 12% of the total body surface.

(7) As an over-all check on the physical procedure and measurements we have calculated the thermal loss or constant for Newton's Law of Cooling. This represents the heat loss by radiation plus convection per unit area of skin per degree of difference between skin and air temperatures. As would be expected the constant is the same for the two sexes and does not change with calorimeter temperature. For radiation alone calculation with the Stephan-Boltzman Law gives much closer results.

In basal metabolism tests with men and women little attention has been paid to the environmental temperature as long as the patient was comfortable. It is, of course, difficult to estimate or measure the actual environmental temperature of a person who is clothed or covered with a blanket. We have not yet made determinations on clothed subjects, and it must be remembered that we have made our measurements only on persons at complete rest, naked and in an environment with minimal air movement. The results, however, indicate that it may be necessary to make changes in the standards of the normal basal metabolism, paying attention to the environmental temperature in the case of women. The lowering of the metabolism in the women in the warm zone speaks for the existence of a "chemical regulation" as described by Rubner for animals. This had never previously been established for the human species although it is generally accepted for animals. The physical regulation of temperature involves changes in the skin, sweating and other physical methods of heat loss and the changes in heat production due to exercise or unconscious tensing of the muscles. The chemical regulation is supposed to be due to some non-physical factor such as a hormone, which changes the level of heat production. The prevailing opinion is that such regulation would appear as the environment began to grow colder and would increase metabolism. Our calorimeter data favor the idea of a factor which lowers the metabolism in warm atmospheres from the standard level established in a cooler zone.

*Summary.*—The men and women whom we have studied under our limited standard conditions showed two points of agreement—Newton's

Law constant and the internal body temperature. In all the other adjustments to changes in the thermal environment the women have a physiological advantage. The comfort zone, in which the heat loss and heat production are equal, extends over a range of about  $6^{\circ}$  for women instead of  $2-3^{\circ}$  for men. The most important factor is the fall in heat production of women in warmer environments. Another factor is the thicker layer of insulation against cold. A third factor is a slightly better adaptation of skin temperature to meet thermal changes in the environment. In cool air women, lightly clad, may be comfortable when the men need woolen clothing. In the warm zone long before the women have started to perspire or even "glow" the men may be covered with beads of sweat. Many of these facts have been suspected for generations but all should be taken into consideration in the practice of air conditioning.

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<sup>9</sup> Mason, E. D., "The Basal Metabolism of European Women in South India and the Effect of Change of Climate on European and South Indian Women," *Jour. Nutrit.*, 8, 695 (1934).

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<sup>11</sup> Du Bois, E. F., *Basal Metabolism in Health and Disease*, Third Edition, Lea and Febiger, Philadelphia (1936).

<sup>12</sup> Hardy, J. D., "Radiation of Heat from the Human Body. An Instrument for Measuring the Radiation and the Surface Temperature of the Skin," *Jour. Clin. Invest.*, 13, 593 (1934).



## ON POSSIBLE CHANGES IN THE SOLAR "CONSTANT"

BY THEODORE EUGENE STERNE, KARL FREDERICK GUTHE AND  
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Communicated May 8, 1940

1. *Introduction.*—In an earlier communication<sup>1</sup> (here called "paper I"), ten periodicities suggested<sup>2</sup> by Abbot were tested for statistical significance, in order to see whether any evidence could be obtained, from measures of the solar constant, of the possible existence of changes in the solar radiation. It was found that three harmonic components (with periods of 7, 8 and 34 months) could be attributed entirely to the effects of ordinary random errors. The other seven (of  $9\frac{1}{2}$ , 11, 21, 25,  $39\frac{1}{2}$ , 46 and 68 months) could not, and therefore they arose either from changes in the solar radiation, or from systematic errors. There was, accordingly, something that it might be profitable to study further.

We describe in the present paper a thorough periodogram analysis of the solar constant, made without regard to Abbot's suggested periods, and with no assumptions about the lengths of any possible periods. We find ten pronounced harmonic components, statistically significant in the sense of their not arising from random errors, but not necessarily real in the sense of corresponding to changes in the solar radiation. Their periods do not correspond in detail to Abbot's. They must exist either (a) in the true values of the solar constant, or else (b) in the systematic errors. It is not definitely determinable from which of the two sources, (a) or (b), they come. Yet the six longest periods, among the ten that are found, are submultiples of 10.2 years and correspond to terms in the Fourier expansion of a variation fundamentally periodic in a 10.2-year period. The average length of the two sun-spot cycles that most closely overlap the 15-year interval of observation is very nearly 10.0 years. The two lengths, 10.2 and 10.0, are equal within the uncertainties of their determination. Therefore, either a fundamental periodicity (not necessarily permanent, but still pronounced over an interval of 15 years), equal in length to the sun-spot period, has somehow crept into the systematic errors, or else there are changes in the amount of the solar radiation. The latter changes must have been periodic during an interval of fifteen years, but they do not have to be of a permanently continuing sort. In this paper we are not interested in the permanency of periodicities. We make use of harmonic analysis, but only in order to see whether we can find evidence, by doing so, for the occurrence of any real changes at all in the amount of the solar radiation.

2. *The Analysis.*—If the observed values of the solar constant contain

any periodically varying part, then that part must be composed of sinusoidal components whose periods are integral submultiples of the period of the variation. Mathematically, the situation is simple. Each periodicity in the values of the solar constant can be represented by a Fourier series, periodic in the period,  $2\pi/s$ , of the periodicity. Besides a trivial constant term, the Fourier series consists of pairs (harmonic components) of the form  $b \sin nst + c \cos nst$  where  $t$  is the time and  $n$  is a positive integer, not zero. It is easy to find, by least squares, the values of  $b$  and  $c$  for a harmonic component having any specified period. This was done, for periods specified by Abbot, in paper I. Abbot does not appear to have chosen his periods through any systematic periodogram analysis, but rather through a process of inspection and trial. We have therefore subjected the material that was examined in paper I to a thorough periodogram analysis of a modified Schuster type. The material consists of 540 ten-day mean values (strictly, "decade" means, over intervals of one thirty-sixth of a year) of the solar constant, covering the interval from 1920 through 1934, and published by Abbot<sup>3</sup> from measures made by the Smithsonian Astrophysical Observatory.

The analysis was carried out for each of 76 trial periods,  $P_u$ , having reciprocals,  $v_u$ , given by the formula

$$v_u = 0.0048 + 0.000926 u \quad (1)$$

in periods per decade, where  $u = -1, 0, 1, 2, 3, \dots, 74$ . The longest trial period was thus about 86 months; it was considered unsafe to use longer trial periods with material covering only 180 months. Equation (1) yields a set of trial frequencies spaced at one-half the resolving power. The shortest trial period was 4.55 months, since the number of different trial frequencies that could conveniently be employed was limited by the eighty columns of the punched-card computing machinery. The observations were numbered serially from 1 through 540. The phase (expressed as a fraction of the trial period) of the  $N$ th observation, according to the trial frequency  $v_u$ , was thus the decimal part of  $Nv_u$ . For each trial period, the observations were classified into ten phase-classes, with phases (rounded to the nearest tenth) running from 0.0 through 0.9. The means,  $M_j$ , of the values of the solar constant in the respective phase-classes were then used as the right-hand members of observational equations of the form

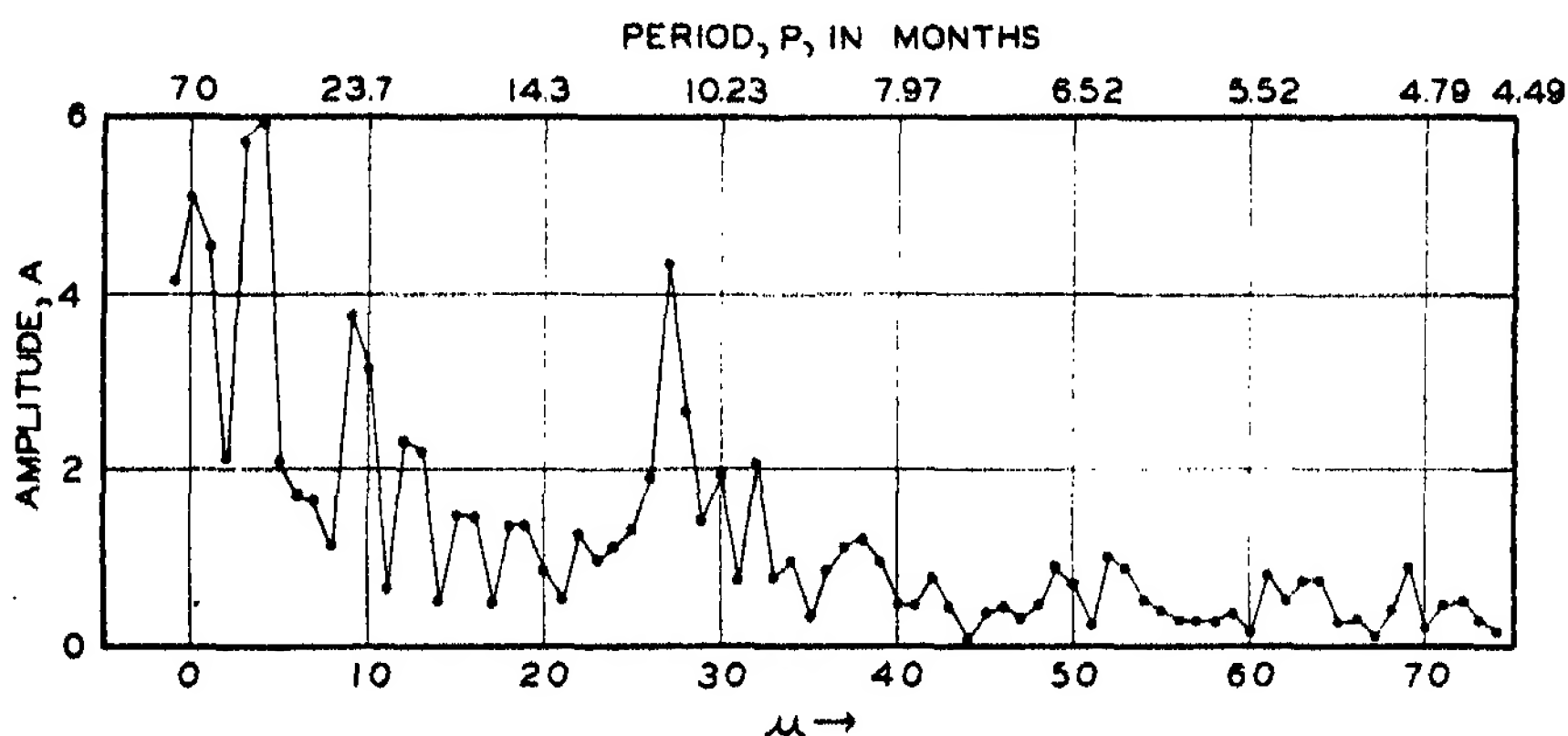
$$a + b \sin 36^\circ j + c \cos 36^\circ j = M_j; \quad j = 0, 1, 2 \dots 9. \quad (2)$$

There were thus ten observational equations for each trial period. The ten equations were assigned equal weights ( $A$ ) in a least squares solution for the coefficients  $a$ ,  $b$  and  $c$ .

It would have been slightly more accurate to have adopted, for the weights ( $B$ ) of the equations, within each least squares solution, the popu-

lations of the respective phase-classes. The populations in question, however, were all close to 54 and it is known, in general, that least squares values are fairly insensitive to changes in the weights. The adopted system of weights (*A*) therefore leads to almost exactly the same values of the coefficients as would the system (*B*), while it facilitates the computations by reducing, to a single form, all of the different least squares solutions.

The results of the preceding analysis are shown in table 1, and in the diagram. Values of the amplitude,  $A = (b^2 + c^2)^{1/2}$ , are listed in the table against the corresponding values of  $u$ , and are plotted against  $u$  in the diagram, which constitutes a periodogram. As it relates to  $u$ , the resolving power is constant and equal to two units throughout the diagram; as it relates to  $P_u$ , the resolving power varies. The straight lines joining the



Periodogram of the ten-day mean values of the solar constant, covering 15 years of observation. The amplitude is in units of  $10^{-3}$  calories per square centimeter per minute.

computed points have been drawn merely to assist the eye, and have no other significance.

Corresponding to any harmonic term having a frequency  $\nu'$ , in the observed values of the solar constant, there exists a certain pattern in the periodogram. It is easy to show\* that the pattern consists of a principal maximum or "peak" at  $\nu = \nu'$ , flanked by secondary maxima located very nearly at  $\nu' \approx 3h/2, 5h/2, 7h/2$ , etc. The ordinate falls to minimum values between them, at  $\nu' \approx h, 2h, 3h, \dots$  etc. The quantity  $h$ , the resolving power, is the reciprocal of the interval of observation, and is here equal to  $0.001852 \text{ decades}^{-1}$ . The heights of the principal and secondary maxima are very nearly in the ratio of  $1 : (2/3\pi) : (2/5\pi) : (2/7\pi) : \dots$ , etc. The pattern is similar to one produced by a diffraction grating having a small number of lines. The occurrence of random errors in the material of course

tends to mask all the peaks to some extent, and the secondary maxima, being smaller than the principal maximum, are the more easily obscured.

It will be noticed that in accordance with equation (1), a change of unity in  $u$  changes the trial frequency  $\nu$  by an amount equal to one-half the resolving power. This spacing was adopted in order to cover the periodogram thoroughly, over the interval tested. With this spacing, each separate principal peak is covered by three or more points.

3. *Results.*—The periodogram contains a considerable number of peaks. In order not to be distracted by the smallest ones, most of which are probably mere statistical fluctuations, let one consider only the twelve maxima on which one or more of the computed ordinates exceed  $10^{-3}$  calories per square centimeter per minute. By interpolation between the computed ordinates, which correspond to integral values of  $u$ , the fractional values of  $u$  may be estimated that correspond to the maxima of these peaks. Such interpolated maxima probably yield the best values of the

TABLE 1  
ORDINATES OF THE PERIODOGRAM IN UNITS OF  $10^{-3}$  CAL./CM.<sup>2</sup> MIN.

$u$	0	1	2	3	4	5	6	7	8	9
-10	...	...	...	...	...	...	...	...	...	418
0	508	457	213	532	594	211	173	164	115	376
+10	316	064	233	221	050	151	145	047	136	136
20	084	052	126	098	113	130	192	436	264	142
30	194	075	206	078	096	035	085	112	121	096
40	047	046	078	043	006	039	042	031	046	088
50	073	023	100	087	054	040	027	028	026	039
60	017	081	052	075	078	026	030	009	042	091
70	020	045	052	026	015	...	...	...	...	...

periods that can be found without the considerable labor of computing additional points on the periodogram, and they are listed in table 2, in the first column. The second column contains the corresponding frequency,  $\nu$ , in periods per decade; the third, the period in months; and the last column, the value of  $P_{bc}$  obtained as in paper I. The number  $P_{bc}$  is the probability, approximately, that statistical fluctuations in the random<sup>†</sup> errors would give rise to a value of  $t_b^2 + t_c^2$  equal to or larger than the value observed for the greatest computed ordinate in the peak. Here  $t_b$  and  $t_c$  are the ratios of  $b$  and  $c$  to their (equal) mean errors, and thus the value of  $P_{bc}$  depends both upon  $A$  and upon the residuals of the observational equations. It is difficult to estimate, accurately, the uncertainty in the interpolated  $u$ 's. If one supposes that such  $u$ 's have uncertainties of the order of 0.5, then the frequencies have uncertainties of the order of 0.0005. The last two digits of the periods in column three are probably doubtful.

The resolving power is two units in  $u$ ; and if one supposes that there are thirty-eight independent trial periods between  $u = -1$  and  $u = 74$ , then one should expect to find only one or two peaks, within this range, with  $P_{bc}$ 's that are equal to or smaller than 0.03, and that arise by chance from the effects of random errors. The expected number is if anything fewer than two, and probably all but two or three of the above peaks must be considered to have statistical significance.

The distribution and heights of the first six peaks in table 2, which appear with the small doubtful seventh peak to form a related family in the periodogram, do not allow the family to be readily explained as being due to the diffraction patterns of a few "principal" peaks. The first six peaks appear to have periods that are closely equal to submultiples of a fundamental period of 368 decades, or 10.2 years. With this fundamental period, a periodicity would have for the  $u$ 's of its 2nd, 3rd, 5th, 6th, 7th and 8th

TABLE 2

$u$	$\nu$ (PER DECADE)	$P$ (MONTHS)	$P_{bc}$
0.14	0.00493	67.6	$7 \times 10^{-4}$
3.64	0.00817	40.8	$4 \times 10^{-6}$
9.31	0.01342	24.8	$1 \times 10^{-40}$
12.43	0.01631	20.4	$3 \times 10^{-3}$
15.44	0.01910	17.45	$1 \times 10^{-3}$
18.50	0.02193	15.20	$4 \times 10^{-3}$
22.23	0.02538	13.13	$3 \times 10^{-3}$
27.09	0.02989	11.15	$9 \times 10^{-25}$
29.80	0.03239	10.29	$7 \times 10^{-6}$
32.01	0.03444	9.68	$3 \times 10^{-3}$
37.76	0.03977	8.38	$2 \times 10^{-3}$
52.36	0.05329	6.26	$3 \times 10^{-3}$

harmonic Fourier components<sup>†</sup> the values 0.69, 3.62, 9.50, 12.43, 15.37 and 18.30, in close agreement with the observed values. The value 368 decades was obtained by least squares from the first six peaks. The peak at 13.1 months does not fit into this scheme, but it is low, its  $P_{bc}$  is the largest in table 2, and it is of no statistical or physical significance. The peak at 17.4 months, although it fits into the scheme, has a  $P_{bc}$  of 0.01, a value hardly small enough to be of statistical significance among so many trial periods. This value of  $P_{bc}$ , however, corresponds to the integer  $u = 15$ ; and it appears from the periodogram that if the ordinate at 15.44 were exactly computed, its  $P_{bc}$  would be considerably smaller. It is therefore considered that this peak is probably statistically significant.

The eleventh Fourier component of a fundamental period of 368 decades would have a  $u$  of 27.11, sufficiently close to the high peak observed at  $u = 27.09$  (11.2 months); but to find so large an amplitude for so high a harmonic would not be likely, and it may not be reasonable to attempt to



relate this large peak to the fundamental period of 10.2 years. The peaks at 10.3 and 9.7 months may be the first and second subsidiary maxima associated with the principal maximum at 11.2 months. The spacing is right for this interpretation, and the plotted heights, it should be remembered, are not the true heights since our analysis has been carried out only for integral values of  $u$ . The small peak at 8.4 months does not appear to have even statistical significance; that at 6.3 months appears to be statistically significant but it may not be related to any of the other peaks in table 2.

The minimum to the left of the peak at 11.2 months appears at  $u = 23$  instead of at  $u = 25.09$  where it ought to be, two units to the left of the maximum. There thus appears to be some evidence, from the periodogram, for the existence of an extra period somewhat longer than 11.2 months. A period of one year would have a peak at  $u = 24.81$ , nearly at the first minimum of the pattern associated with an 11.2-month period. A significant  $P_{bc}$  was obtained for this annual periodicity in paper I. Perhaps an annual period (a systematic error arising from the atmosphere) contributes to the periodogram a peak that merges with the diffraction pattern of the 11.2-month period to produce the observed peak as a resultant; if this is true then the interpolated value of 11.2 months may be slightly in error.

To sum up, there appears to be a family of statistically significant harmonic components with periods of about 68, 41, 25, 20, 17.4 and 15.2 months. These periods are equal to submultiples of 123 months, closely enough for it to be possible to regard them as the Fourier components of a fundamental period of 123 months. There are also significant harmonic components which, whether related to the preceding periods or not, have periods of about 11.2 and 6.3 months. Significant periods, indicated at 10.3 and 9.7 months, may possibly be "diffraction" phenomena associated with the pronounced period at 11.2 months. There is some evidence for a possible annual term. The present list of periods differs from Abbot's. His 68-month period is the same as ours, but his 46-month period falls upon the rising portion of the 41-month peak and does not appear to have a separate existence. His  $39\frac{1}{2}$ -month period appears in our list as 41 months. His 34-month period corresponds to a minimum in the periodogram and does not seem to exist. His 25-month period is in our list, and his 21-month period is our 20-month period. Our 17.4 and 15.2-month periods are absent from his list; his 11-month period is our 11.2-month period; our 10.3-month period is missing from his list; and his 9.75-month period is our 9.7-month period. His 7 and 8-month periods come close to minima and do not appear to exist, while our 6.3-month period is missing from his list.

That six of the ten components should appear to be overtones of a funda-



mental periodicity of about 10.2 years suggests, to some extent, a dependence of the solar radiation itself upon the phenomena that underlie the sun-spot, and other solar periods. Over the 15-year interval during which the observations of the solar constant were obtained, the average length of two sun-spot cycles appears to have been about 10.0 years. As pointed out in the *Introduction*, this implies that unless a fundamental period equal to the sun-spot period has somehow crept into the systematic errors then there must be changes in the true solar constant itself.

Dr. Abbot has kindly informed us that he plans to publish, shortly, values of the solar constant from 1920 through 1939—values computed by improved methods of reduction. The new systematic errors may be smaller, and in any case should be different. It is planned to obtain a periodogram of the new material when it is published. A comparison of that periodogram with the present one may possibly make more certain the origin of the periodicities—whether they come from the systematic errors, or from real changes in the solar radiation. Until then, we wish to suspend further judgment on this most important question.

The authors wish to thank the Harvard Graduate School of Business Administration, and in particular Professor Theodore H. Brown, for very kindly allowing us to use their punched-card computing machinery, of the Hollerith type.

4. *Summary.*—A thorough periodogram analysis has been made of five hundred and forty ten-day mean values of the solar constant, published by Abbot. Seventy-six equally spaced trial frequencies have been used, corresponding to periods between 86 and 4.6 months. The analyzed material contains harmonic components (statistically significant in the sense of not arising from random errors, but not necessarily real in the sense of corresponding to changes in the solar constant), with amplitudes greater than  $10^{-3}$  cal./cm.<sup>2</sup> min., at 68, 41, 25, 20, 17.4, 15.2, 11.2 and 6.3 months. The last digits are uncertain. Two other peaks, at 10.3 and 9.7 months, may perhaps be subsidiary maxima associated with the 11.2-month component. The new list of periods differs in detail from Abbot's, and the six longest members appear to be the Fourier components of a fundamental periodicity having a length of 10.2 years. Within the accuracy of their determinations, this last period, and the average sun-spot period at the time of the observations, appear to be equal. The equality suggests the occurrence of some real changes in the solar constant, but pending the publication by Abbot of revised material, this point should not be regarded as settled.

\* . . . by considering, analytically, the nature of the periodogram that would be obtained from a quantity  $a + b \sin 2\pi v'N + c \cos 2\pi v'N$ .

† The measures of the solar constant may be considered to consist of the solar constant, plus random errors, plus systematic errors. By random errors, here and in paper I,

errors are referred to that may be considered to have been drawn independently and at random from some parent population whose mean is zero. Errors of all other sorts are called *systematic*. Hulme,<sup>4</sup> in a short paper with much of which we agree, appears to misunderstand this fairly conventional use of the expression *random errors*. Correlated errors, for us, are systematic errors.

† There is, of course, no reason why the 4th component should not be small.

<sup>1</sup> Sterne, *Proc. Nat. Acad. Sci.*, 25, 559 (1939).

<sup>2</sup> Abbot, *Smithsonian Misc. Coll.*, 94, No. 10 (1935).

<sup>3</sup> Abbot, *Ibid.*, p. 12.

<sup>4</sup> Hulme, *Observatory*, 63, 101 (1940).

## ON SOLAR FACULAE AND SOLAR CONSTANT VARIATIONS

BY HENRYK ARCTOWSKI

SMITHSONIAN INSTITUTION

Read before the Academy, April 23, 1940

On January 4, 1845,<sup>1</sup> experimenting with a thermopile of Ruhmkorff Professors Henry and Alexander of Princeton<sup>1</sup> made "twelve sets of observations, all of which, except one, gave the same indication, namely, that the sunspots emitted less heat than the surrounding parts of the luminous disc."

Exact measurements of the thermal conditions of sun-spots were made by S. P. Langley in 1874 and 1875.<sup>2</sup> He found that "taking the mean thermal photospheric radiation in the spots vicinity as unity, the mean umbral radiation is  $0.54 \pm 0.005$ , the mean penumbral  $0.85 \pm 0.01$ ." Let us notice also that the ratios of the radiation from the umbrae and the neighboring photosphere, observed by W. E. Wilson<sup>3</sup> in 1893 and 1894, varied between 0.29 and 0.85, and that E. B. Frost,<sup>4</sup> comparing Langley's observations, made about three years after a solar maximum, with his own, made about two years after a minimum, writes that "it is impossible to assert that the thermal conditions of spots (and perhaps of the photosphere and atmosphere) are invariable during the eleven-year period of solar activity."

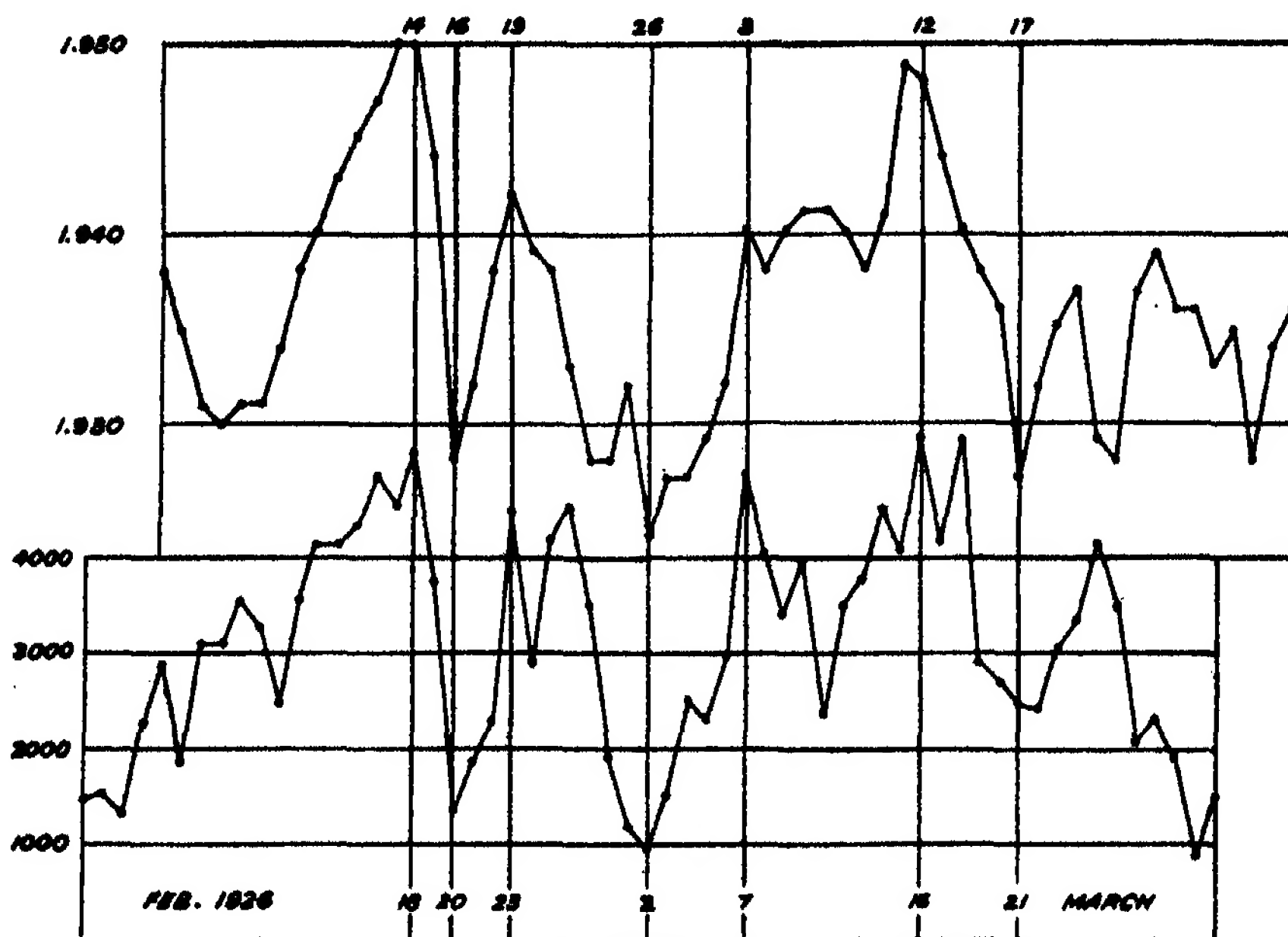
The solar faculae were correctly described long ago by Christ Scheiner in his "*Rosa Ursina . . .*" published in 1626, and by I. B. Ricciolo, as well, in his "*Almagestum Novum . . .*" published in 1651.

Secchi noticed that when a facula is observed at the edge of the solar disc, the chromosphere nearby extends higher. J. Fényi,<sup>5</sup> who cites this observation, writes that in the spectrum of faculae the lines of prominences appear very bright, the *K* line in particular. This fact allowed Hale and Deslandres to photograph the distribution of faculae all over the solar disc. Fényi considers the faculae as being redescending gas masses of the ascending prominences. He thinks it is because of adiabatic compression that

the temperature of faculae should be higher than that on the corresponding levels of the solar atmosphere.

If, therefore, the spectrobolometric measurements of C. G. Abbot, F. B. Fowle and L. B. Aldrich, giving the mean radiation along the radius of the solar disc,<sup>6</sup> are accepted as standard, local deviations on the surface of the sun should be admitted, *a priori*, in particular over the areas occupied by faculae.

Daily contrast numbers of brightness, solar center-limb, observed by Dr.



Variations of the solar constant and of areas of solar faculae

FIGURE 1

Daily solar constant values for February and March, 1926, and areas of faculae.

Abbot and his co-workers in 1913 and 1914, compare well, in their variations, with those of solar constant values.<sup>7</sup>

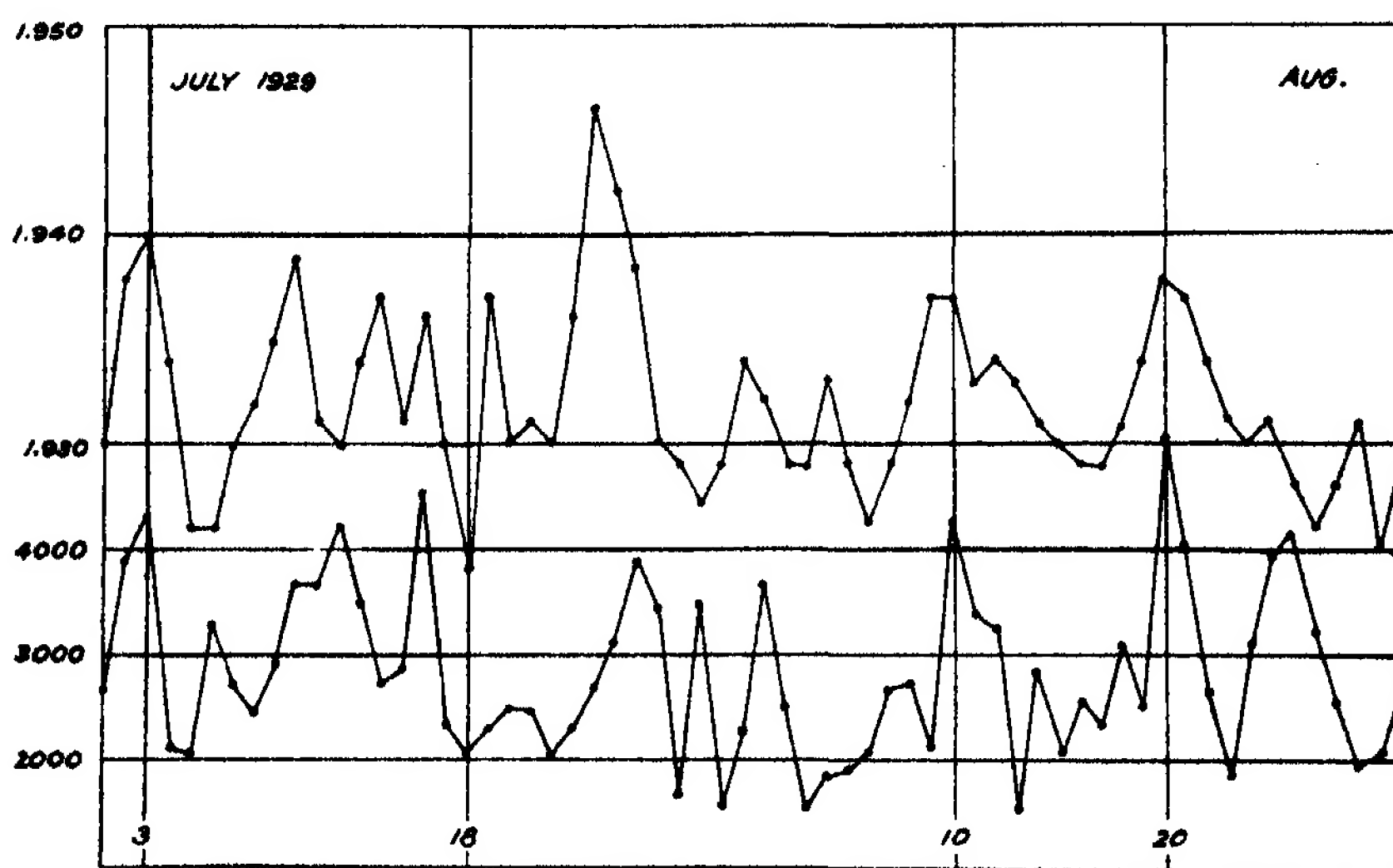
It was these facts which, in 1915, led me to the study of the variations of the daily areas of faculae, and their monthly means,<sup>8</sup> as recorded in the Greenwich photo-heliographic results.<sup>9</sup>

I found that, during the approximately 11-year period of sun-spots, there are five maxima of the quotients of faculae and umbrae areas and that the frequency variations of sun-spots should be considered as a subordinate manifestation of the variations of faculae.<sup>10</sup>

Dr. Abbot's researches on solar constant data led him to the conclusion

that "an increase of 0.07 calory per square centimeter per minute in the solar constant accompanies an increase of 100 sunspot numbers," while my researches on the fluctuations of the solar constant led me to believe that sun-spots should be considered as only one of the acting factors, probably less important than the faculae or other unmentioned solar phenomena on which the areas of faculae depend.<sup>12</sup>

The fact is that F. E. Fowle, comparing the daily data of solar constants of radiation, observed during March, April and May, 1920, with the areas of calcium flocculi, measured at the del Ebro Observatory, noticed an excellent agreement of their variations.<sup>13</sup> Besides, H. H. Clayton ascertained



Solar constant and solar faculae

FIGURE 2

Solar constants and faculae, July and August, 1929.

that from July, 1918, to December, 1921, maxima of faculae coincided with maxima of solar radiation, and beginning in May, 1924, he has made forecasts of solar constants from the visual observations of sun-spots and faculae.<sup>14</sup>

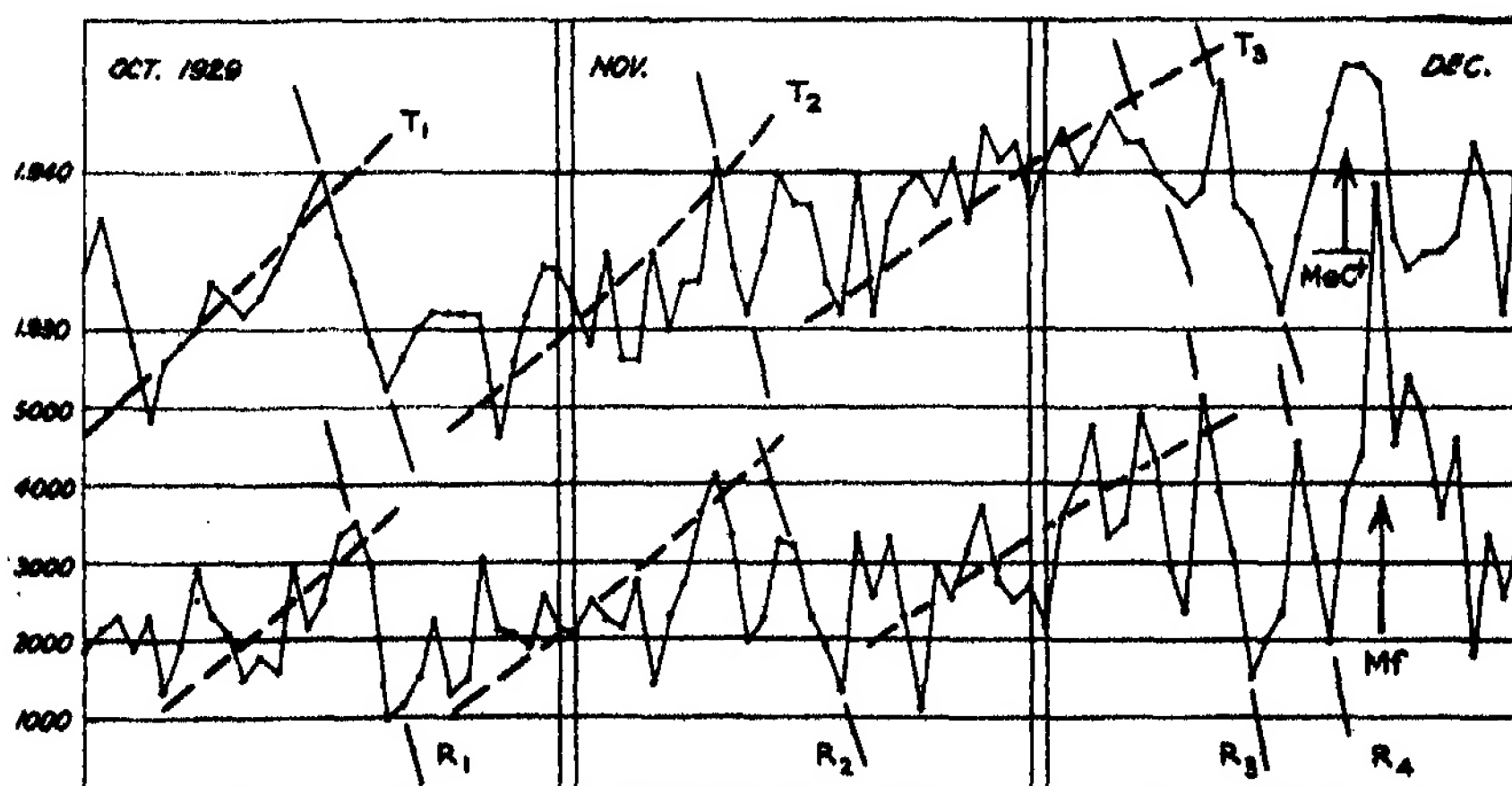
I began my work at the Smithsonian, last December, by studying the daily values of preferred solar constants observed from 1926 to 1930.<sup>15</sup> Dr. Abbot has given the following grades to the tabulated data: *S* satisfactory, *S*— not quite satisfactory, *U* unsatisfactory, *U* + better than unsatisfactory and *U*— very unsatisfactory. Four hundred ninety-four of these values, approximately only one-fourth of the total number of observations, were considered perfectly satisfactory. It follows that the es-

tablishment of many solar constant observatories would be advisable if continuous solar constant data are ever to be used in daily weather forecasts, in order to avoid unsatisfactory data which are due exclusively to the temporary local unfavorable atmospheric conditions.

At present let us examine the question of the cause of solar constant variations.

Leaving aside the eventual correlations between sun-spots (areas of umbrae) and the Smithsonian solar constant values, which lead to contradictory results, as I showed long ago, it is only the Greenwich data of areas of faculae, corrected for foreshortening, that will be taken into consideration.

Comparing the diagrams of areas of faculae, expressed in millionths of



Discontinuous trends in solar constant and solar faculae

FIGURE 3

Solar constants and faculae, October, November and December, 1929.

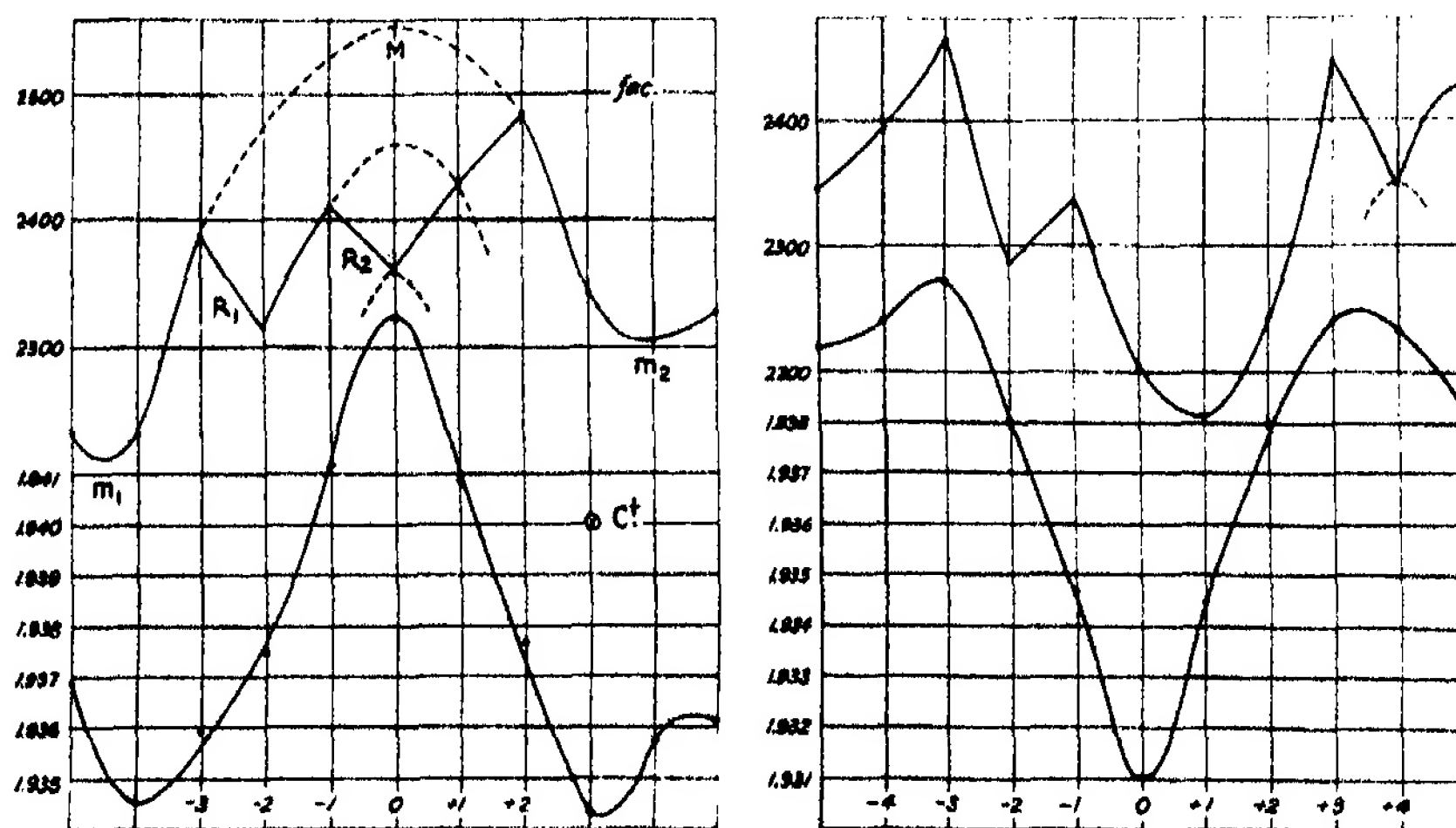
the sun's visible hemisphere, with those of the solar constant values, I noticed that, in order to observe a more or less satisfactory agreement, we must admit that in most cases the maxima and minima of solar radiation precede those of faculae. Taking the daily data of February and March, 1926 (Fig. 1), we must admit a close agreement between the radiation minimum on February 16th and the minimum of faculae on the 20th. But this difference, of four days' advance, is far from being general. Maxima on July 3, August 10 and 20, 1929, for example (Fig. 2), occur simultaneously on both curves.

The months of October, November and December, 1929, may be taken as another example showing that not only the maxima and minima of both curves may correspond to each other, but also that characteristic inter-

ruptions in the trend may be observed, such as could happen only in the case of cause and effect. On figure 3 the upward trend  $T_1$  repeats itself in  $T_2$  and  $T_3$ , after the ruptures  $R_1$  and  $R_2$  and the well-pronounced maxima  $M \odot C'$  and  $Mf$  display perfectly the cause and its effect, in this case belated by two days.

It seems evident that if the curves are not more similar than they are, many not perfect or even unsatisfactory solar constant values may be the cause of it.

These comparisons justified taking into consideration mean values. Therefore, for the years 1926 to 1930, I took all the maxima of solar con-



Time relations between maxima and minima in the solar constant and solar faculae

FIGURES 4 AND 5

Means of faculae and solar constants for the 5 days before and the 5 days after the dates of 72 selected days of maxima and 82 days of minima of solar constants.

stant values, preceded by increasing and followed by decreasing values for at least three days before and after. The same was done for the minima. The means of the 72 series of figures for the maxima and 82 for the minima give the diagrams on figures 4 and 5. Now, taking the values of the faculae for the same dates, we find (Fig. 5) that the mean minimum of faculae occurs one day after the mean minimum of solar radiation. The time difference between Greenwich and Washington makes it more than a day.

In the case of the mean maxima there is a difference of two days (Fig. 4). But considering the lines  $R_1$  and  $R_2$ , between the third and the second days before the maximum of the solar constant, as discontinuities of a regular variation  $m_1 M m_2$ , we would have a coincidence of maxima. Therefore this



diagram induces the hypothesis that the solar atmosphere reacts against a full development of the faculae maximum, as it should have developed under the influence of the maximum of radiation. Nor is the minimum of faculae as deep as it should be.

In other words, the solar constant variations are not due to faculae, but the variations of the extent of faculae are due to the same variations of photospheric radiation as those of the solar constant.

A certain similarity to what occurs in our terrestrial atmosphere seems obvious: rain and hail subtract water from a thunderstorm cloud which is formed under the influence of strong ascending air currents; and, as we know, in the case of a minimum of such air chimneys, clouds may still be formed.

Do the solar constant variations affect the meteorological phenomena observed in our atmosphere?

For years Dr. Abbot has advocated such a thesis.

Meteorologists, in general, had doubts. I also was not entirely convinced, before the last few months of research work. But now I have the necessary data to prove, at least to my own satisfaction, not only that the processes in the solar photosphere—which produce changes in the extent of faculae, observed from day to day or from one group of days to another—directly affect the measured values of the solar constant, but also that in our atmosphere the changes of solar radiation, expressed by the figures of solar constant variations, are the direct cause of anomalies in the distribution of temperature and of all the complexity of the meteorological phenomena depending on temperature anomalies.

<sup>1</sup> *Proc. Am. Phil. Soc.*, 4, 175, Philadelphia (1847).

<sup>2</sup> *M. N. R. Astr. Soc.*, 37, 5, London (1877).

<sup>3</sup> *M. N. R. Astr. Soc.*, 55, 460, London (1895).

<sup>4</sup> *A. N.*, 130, 146, Kiel (1892).

<sup>5</sup> *A. N.*, 140, 300, Kiel (1896).

<sup>6</sup> *Ann. Astroph. Obs. Smiths. Inst.*, 3, 159, Washington (1913).

<sup>7</sup> *Smiths. Misc. Coll.*, 66, No. 5, 18–19, Washington (1917).

<sup>8</sup> *C. R.*, 161, 434, 485, Paris (1915).

<sup>9</sup> "Results of measures made . . . of Photographs of the Sun at Greenwich, the Cape and Kodaikanal in the year . . ."

<sup>10</sup> *C. R.*, 163, 665–667, Paris (1916).

<sup>11</sup> *Ann. Rept. Smiths. Inst. for 1913*, p. 182.

<sup>12</sup> *Loc. cit.*, p. 666.

<sup>13</sup> C. G. Abbot, *Smiths. Misc. Coll.*, 77, No. 5, 23, Washington (1925).

<sup>14</sup> *Smiths. Misc. Coll.*, 77, No. 6, 53, Washington (1925).

<sup>15</sup> *Ann. Astroph. Obs. Smiths. Inst.*, 5, 279–285, Washington (1932).

*THE COURSE OF THIAMIN METABOLISM IN MAN AS  
INDICATED BY THE USE OF RADIOACTIVE SULFUR\*.<sup>†</sup>*

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When supplementary thiamin (Vitamin B<sub>1</sub>) is ingested or injected, the amount which appears in the urine during the succeeding twenty-four hours usually increases, but the total additional excretion always falls short of the supplement, even when the vitamin is injected and there can be no question of incomplete absorption. We have sought information on this unaccounted-for moiety by synthesizing thiamin from sulfur which contains the radioactive isotope S<sup>35</sup> (designated as B<sub>1</sub><sup>\*</sup>) and by following, after injection of the B<sub>1</sub><sup>\*</sup>, the excretion of the radiosulfur (S<sup>\*</sup>) in the urine and feces and the excretion of total free B<sub>1</sub> in the urine.

Radiosulfur was prepared by bombarding elementary sulfur with 7.5 MEV. deuterons, and thiamin bromide hydrobromide containing S<sup>35</sup> was synthesized from the bombarded sulfur.<sup>1</sup>

The half-life of radiosulfur is about 88 days and it emits negative electrons with a maximum energy of 0.107 MEV.<sup>2</sup> This long half-life makes extended observations possible; some samples showed measurable activity 18 months after the radiosulfur had been prepared.

The radioactive vitamin was injected intramuscularly in two series of experiments; in the first series the subject, a young man in good health, was on a vitamin B<sub>1</sub>-free diet for 36 days prior to the first injections and for the 15 days of the experimental period; in the second series the same subject was on a normal diet. Essentially the same results were obtained in both series.

Free (unphosphorylated) vitamin B<sub>1</sub> was determined in the urine by a modification of the thiochrome method. The urinary sulfur compounds were fractionated into the inorganic sulfate, ethereal sulfate and neutral sulfur components, the sulfur of each component was converted into elementary sulfur,<sup>3</sup> and the radioactivity of the sulfur was then measured quantitatively by comparison with a standard sample of the same radiosulfur. In the feces only the total radiosulfur was determined.

The radioactivity measurements were made with open, coincidence Geiger counters. The sulfide-coated copper counter tubes and the samples were enclosed in a large partially evacuated bell jar, provided with an externally operated arrangement for moving the samples successively to a definite position in front of the counters. The standard Rossi coincidence

circuit was used in the amplifiers; these had time constants of  $3 \times 10^{-4}$  seconds. It was possible to measure samples as weak as 3% of the background by making very long runs.

Separate experiments showed that  $B_1^*$  added to urine does not exchange its radiosulfur with the inorganic or ethereal sulfur compounds; the  $S^*$  was quantitatively recovered from the neutral sulfur fraction.

Figure 1 shows the daily excretion of radiosulfur (all forms) in the urine and feces after a daily intramuscular injection of 16 mg. of  $B_1^*$  for four

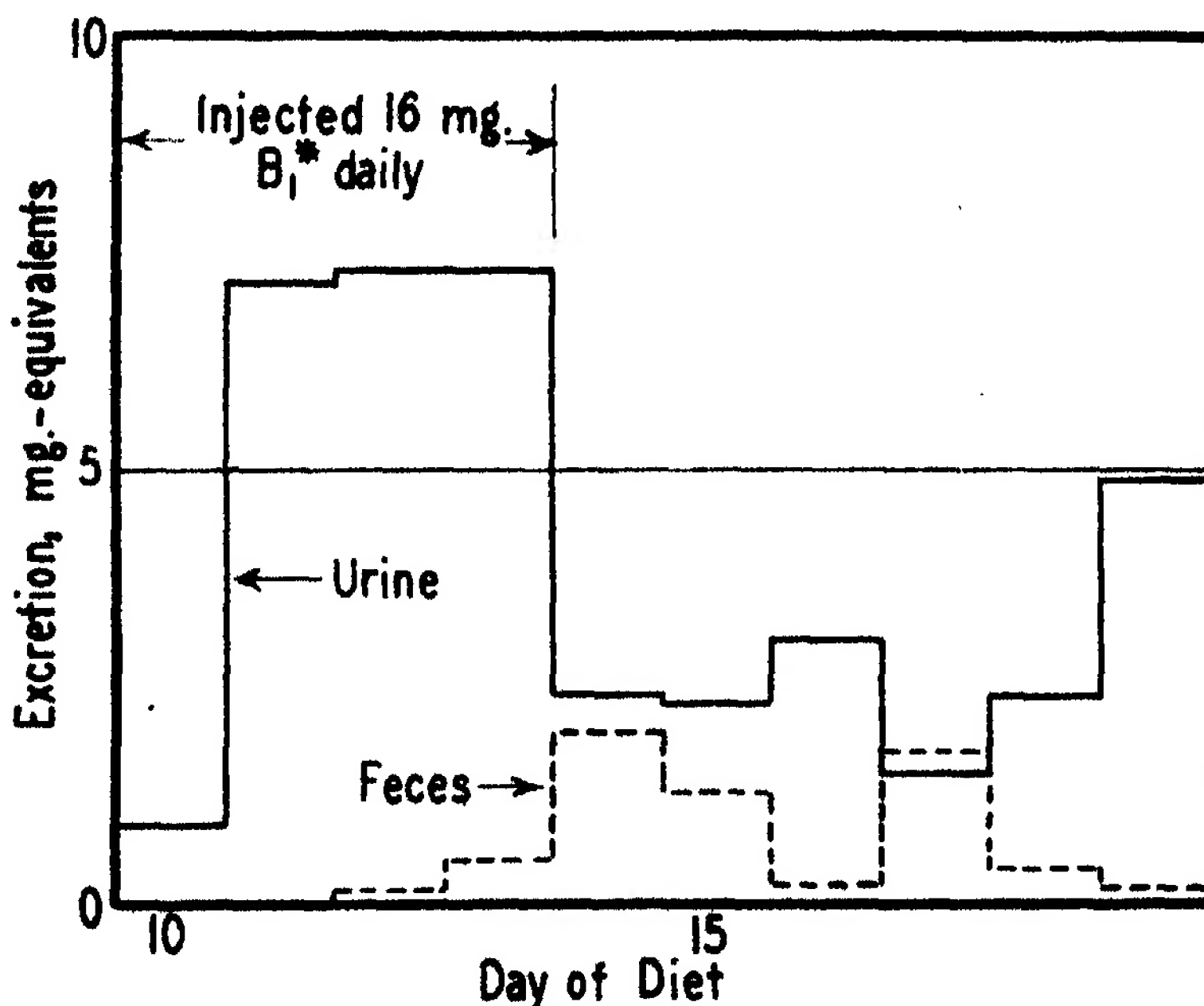


FIGURE 1

The daily recovery of radiosulfur (calculated as mg. of vitamin  $B_1$ ) after intramuscular injection of  $B_1^*$ ; normal diet.

days, while the subject was on the normal diet. Six days after the last injection a total of 61% of the injected radiosulfur had been recovered from the urine and 11% from the feces; 28% of the injected material remained unaccounted for. It is clear from the low and diminishing excretion in the feces that the urine is the major excretory medium of parenterally administered  $B_1$  and its decomposition products.

Figure 2 shows the daily excretion of free thiamin in mg. and of the radiosulfur present in the neutral sulfur fraction expressed in terms of mg. of  $B_1^*$ . If only pure  $B_1^*$  is present in urine these two should be the same;

consequently the excess of thiamin over radiosulfur shown in the figure indicates excretion of thiamin already present in the body. Since the total amount of  $B_1$  in the blood in this case could hardly have exceeded 1 mg.<sup>4</sup> the excess of 7.5 mg. of non-radioactive vitamin in the urine on the first day of the injections must have come from the tissues. This indicates that the injected vitamin interacts very rapidly with that preëxisting in the tissues.

Although the whole of the neutral radiosulfur in the urine during the injection period shown in the figures may have been vitamin  $B_1$ , this is im-

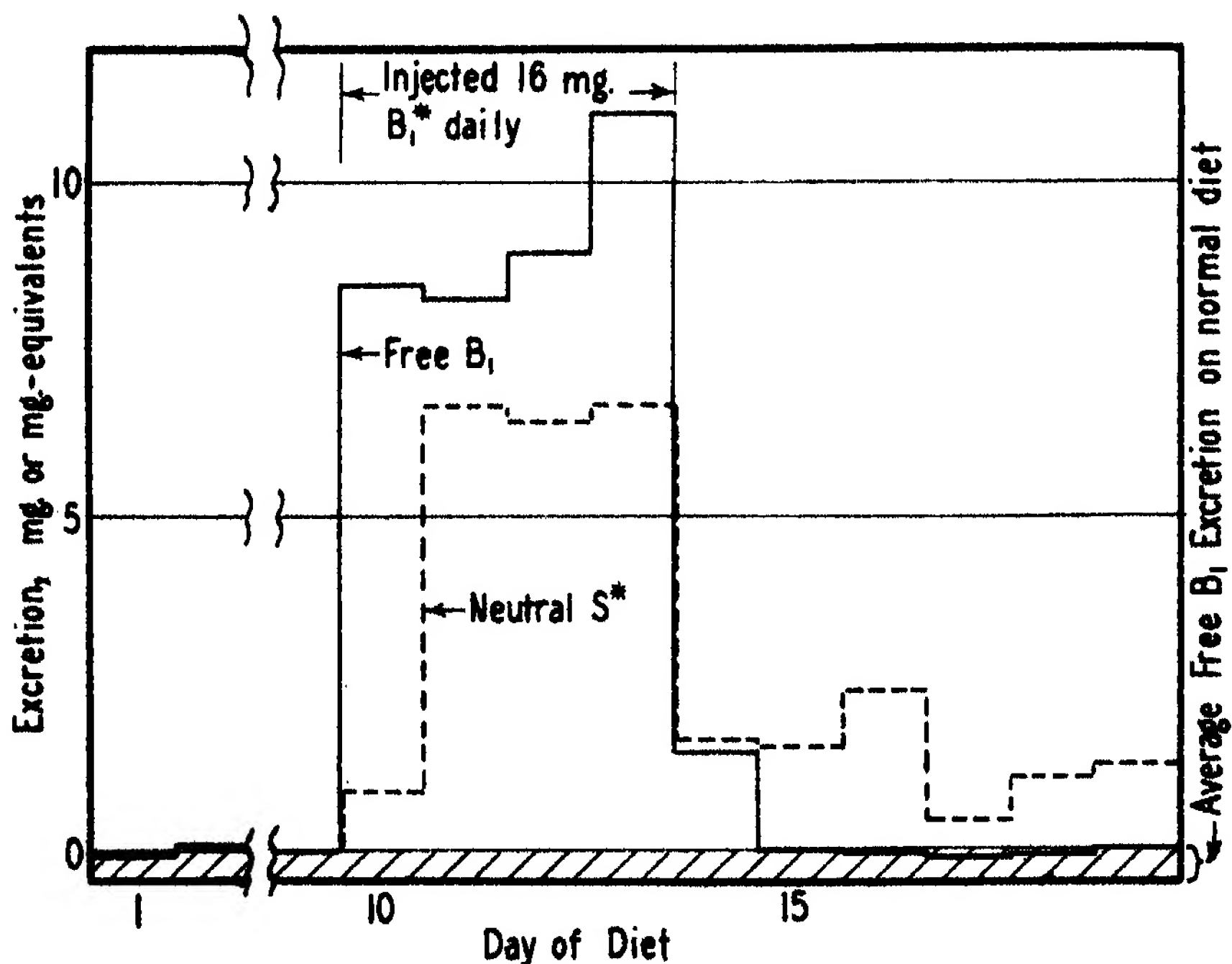


FIGURE 2

The daily excretion of neutral radiosulfur and free thiamin in the urine after intramuscular injection of  $B_1^*$ ; normal diet. The radiosulfur has been calculated in terms of mg. of vitamin  $B_1$ .

probable in view of the findings on the later days. The difference between the total free  $B_1$  and the total neutral radiosulfur is certainly the minimum amount of preëxisting vitamin which was displaced from the tissues.

During the six days following the termination of the radiothiamin injections the neutral radiosulfur in the urine exceeded the thiamin excretion. The difference between the neutral radiosulfur and the free  $B_1$  sulfur is the minimum amount of neutral sulfur-containing decomposition products of the thiamin which was excreted. The continued excretion of radio-

sulfur is evidence that tissue thiamin is continuously undergoing destruction at a rapid rate.

These features were shown even more clearly in the series of experiments when the subject was on the  $B_1$ -free diet and 2.7 mg. of  $B_1^*$  were injected daily from the 37th to 46th days, inclusive, of the  $B_1$ -free diet (Fig. 3). Thiamin excretion in the urine increased immediately, but no radiosulfur was detected in the urine for the first two days (less than 20  $\gamma$ ). The entire increment in the urine came from preëxisting thiamin in the tissues which was displaced by the injected material, again indicating that injected  $B_1$

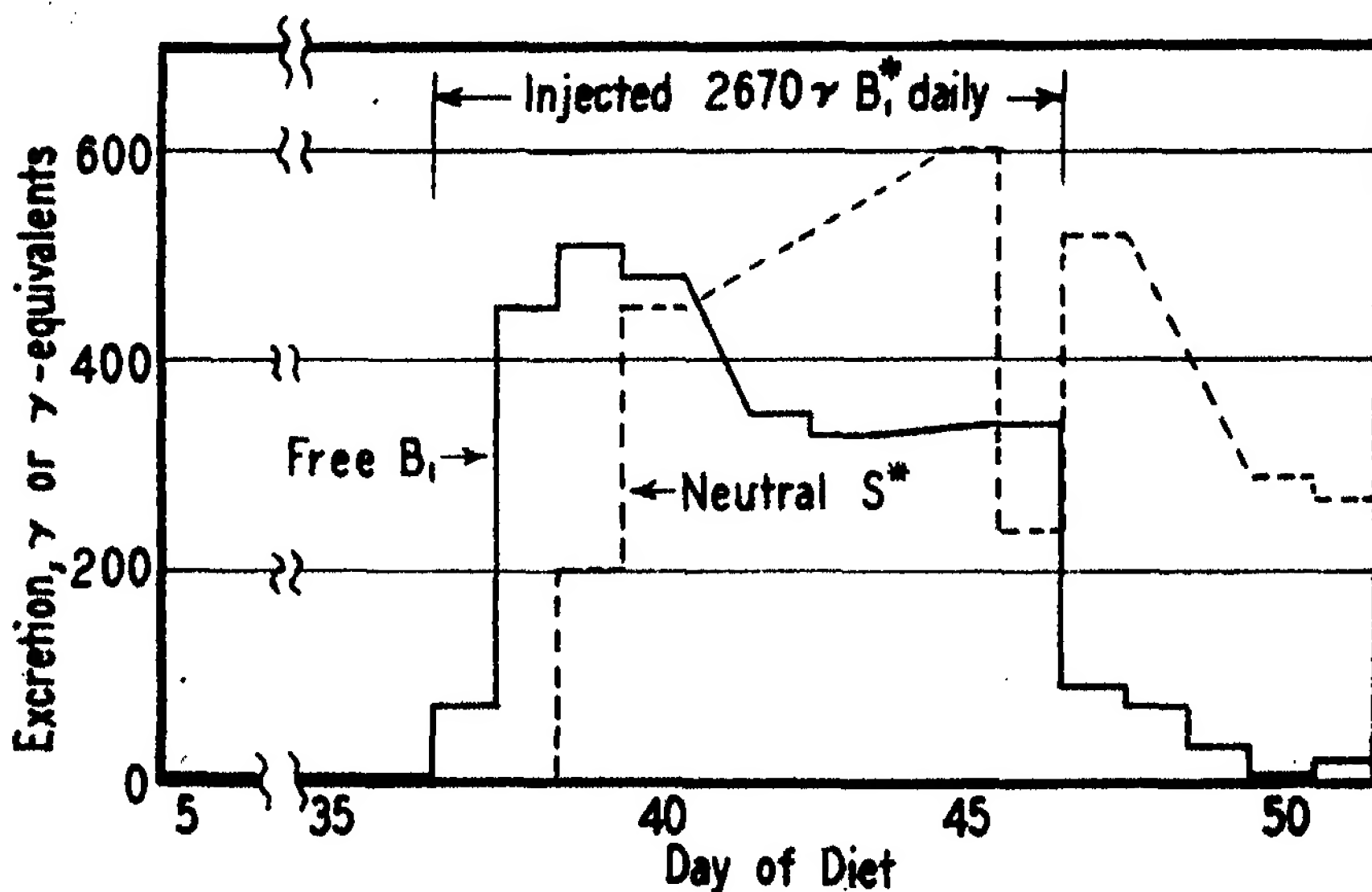


FIGURE 3

The daily excretion of neutral radiosulfur and free thiamin in the urine after intramuscular injection of  $B_1^*$ ;  $B_1$ -free diet. The radiosulfur has been calculated in terms of mg. of vitamin  $B_1$ .

rapidly enters the tissues from the blood. Since no thiamin was detectable in the urine for 30 days prior to the injections, the total quantity in the blood must have been less than 0.2 mg. For a short time at least after the injection of 2.7 mg., the ratio of radiothiamin to normal thiamin in the blood was probably greater than 1. If the excreted vitamin is an "overflow" moiety excreted before it enters the tissues, it should have been accompanied by a corresponding radiosulfur activity, but this was not found to be the case. The injected thiamin, therefore, must have entered the tissues very rapidly and interacted with the considerable quantities of thiamin already present.

Table 1 is a summary of some of the data illustrating this feature of thiamin metabolism. It is interesting that any free thiamin was displaced from the tissues of a subject who had been for 36 days on a B<sub>1</sub>-free diet.

TABLE 1

THE DISPLACEMENT OF PREEXISTING THIAMIN FROM THE TISSUES BY ADDITIONAL THIAMIN INJECTED INTRAMUSCULARLY

DIET	INJECTION AND EXCRETION PERIOD (DAYS)	THIAMIN INJECTED (MG.)	MINIMUM AMOUNT OF PREEXISTING THIAMIN EXCRETED (MG.)
B <sub>1</sub> -Free	3	8	0.8
Normal	1	16	7.5
Normal	4	64	16.0

The neutral sulfur and thiamin data in the two experiments are summarized in figure 4 which shows the difference between the thiamin and the neutral radiosulfur excretion. During the injection period the deficit of neutral radiosulfur indicates the minimum amount of preexisting vitamin displaced from the tissues; the excess of neutral radiosulfur found in the urine after stopping the injections represents destroyed vitamin.

Figure 5 summarizes the urinary excretion of radiosulfur as inorganic sulfate; the radioactive sulfur appeared in this form in small quantities on the second day of the injections, and increased to considerable amounts by the end of the experiment, which is proof of the destruction of the injected vitamin and oxidation of the thiazole ring. The SO<sub>4</sub>\* from this oxidation mixes with the large amount of SO<sub>4</sub> in the body;<sup>6</sup> the excretion of the radiosulfur is therefore delayed. The oxidation of the thiazole ring was therefore probably much more extensive than is indicated by the amount of SO<sub>4</sub>\* in the urine in the first few days after beginning the injections.

The amounts of radiosulfur found as ethereal sulfate were in most cases very close to the experimental error of the radioactivity measurements; only a very small fraction of the radiosulfur appears in this form, and it may have arisen from exchange with the inorganic sulfate.

The extensive destruction of B<sub>1</sub> in the body is indicated in table 2.

TABLE 2

DESTRUCTION OF INTRAMUSCULARLY INJECTED THIAMIN

	TOTAL RECOVERY OF S* FROM B <sub>1</sub> * (% OF TOTAL INJECTED)		PER CENT OF RECOVERED S* IN THE URINE WHICH REPRESENTS DESTROYED VITAMIN		
	FECES	URINE	AS NEUTRAL S COMPOUNDS	AS INORGANIC SULFATE	TOTAL
B <sub>1</sub> -Free	..	26	21	21	42
Normal	11	61	18	25	43

*Summary.*—1. There is a rapid interaction of injected B<sub>1</sub> with that present in the blood and tissues.



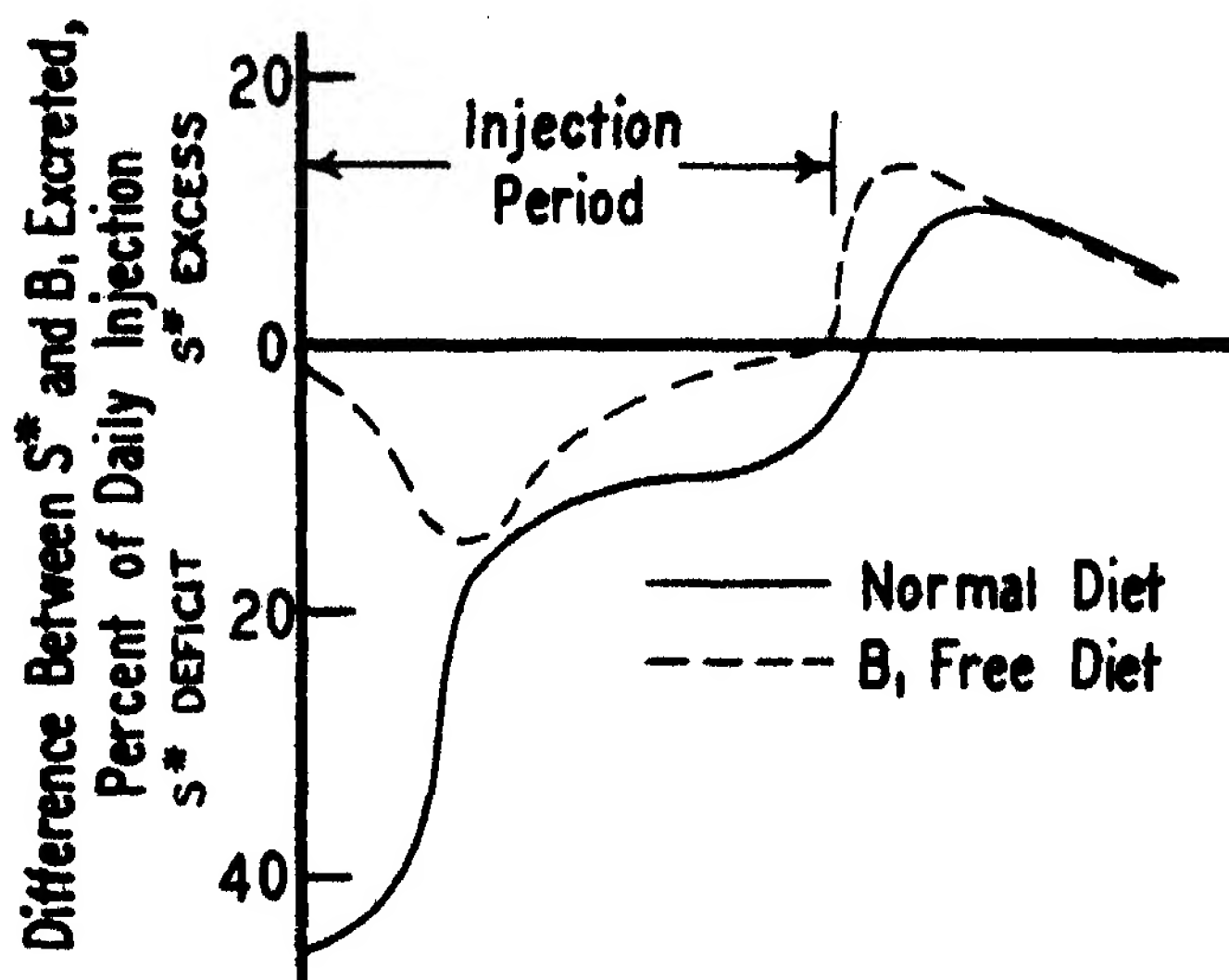


FIGURE 4

The difference between neutral radiosulfur and thiamin excretion after the injection of  $B_1^*$ .

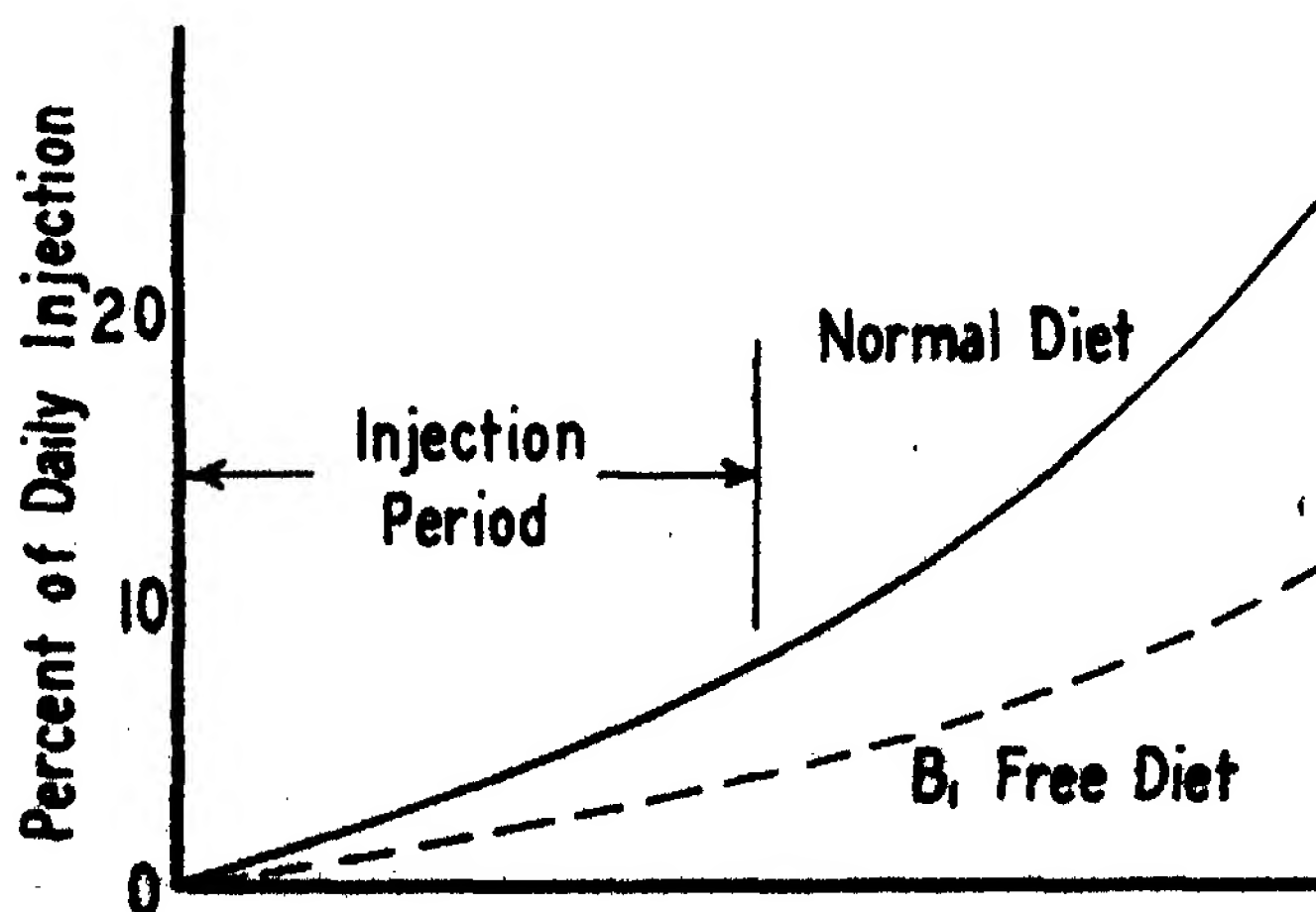


FIGURE 5

The urinary excretion of radiosulfur as inorganic sulfate after the injection of  $B_1^*$ .

2. The displacement of preëxisting thiamin by injected thiamin demonstrates that a significant amount of the vitamin remains in the tissues even after 36 days of a B<sub>1</sub>-free diet; larger quantities are present under normal nutritional conditions. This does not imply that the amount retained after a prolonged B<sub>1</sub>-free diet is an adequate protective amount of this vitamin.

3. The metabolism (interchange and destruction) of vitamin B<sub>1</sub> is rapid and thus resembles that of the main metabolites—protein, fat and carbohydrate.

4. The rapid destruction of thiamin yields in the urine neutral sulfur compounds and inorganic sulfate.

5. The losses incurred by excretion and destruction are inevitable in the maintenance of a physiologically adequate concentration of thiamin and cocarboxylase in the blood and tissues.

\* This work was aided by grants from the National Research Council, the Research Corporation, the Hixon Fund and the Globe Mills Fund (California Institute of Technology).

† This paper was presented at the meeting of the American Society of Biological Chemists in New Orleans, March 14–16, 1940.

<sup>1</sup> Cf. E. R. Buchman, *Jour. Am. Chem. Soc.*, **58**, 1803 (1936); J. K. Cline, R. R. Williams and J. Finkelstein, *Ibid.*, **59**, 1052 (1937).

<sup>2</sup> W. F. Libby and D. D. Lee, *Phys. Rev.*, **55**, 245 (1939).

<sup>3</sup> R. A. Cooley, Don M. Yost and Edwin McMillan, *Jour. Am. Chem. Soc.*, **61**, 2970 (1939).

<sup>4</sup> Robert Goodhart and H. M. Sinclair, *Jour. Biol. Chem.*, **132**, 11 (1940).

<sup>5</sup> Henry Borsook, Geoffrey Keighley, Don M. Yost and Edwin McMillan, *Science*, **86**, 525 (1937).

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## RADIOACTIVE CARBON IN THE STUDY OF RESPIRATION IN HETEROTROPHIC SYSTEMS

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Communicated May 15, 1940

It is now well established<sup>1</sup> that the presence of small amounts of CO<sub>2</sub> are indispensable for the growth of many types of heterotrophic organisms. Also it has been reported<sup>2</sup> that the reduction of methylene blue by certain bacteria is dependent upon traces of CO<sub>2</sub>. It appears that some of the experiments we have been doing with radioactive carbon have direct bearing on these interesting results.

Several heterotrophic (non-photosynthetic) systems, namely, yeast (bakers'), *B. coli*,<sup>3</sup> ground plant (barley) roots, ground liver (rat) tissue, etc., have now been found to assimilate small quantities of radioactive carbon as  $\text{CO}_2$ . This assimilation is inhibited by the presence of  $\text{HCN}$ . Since in the respiratory processes of these heterotrophic cells there is a net production of  $\text{CO}_2$  it is clear that a  $\text{CO}_2$  assimilation can best be studied directly by isotopic tracer methods using either stable ( $\text{C}^{13}$ ) or radioactive ( $\text{C}^{11}$  and  $\text{C}^{14}$ ) carbon.

It is also apparent that the presence of radiocarbon in oxidation states lower than +4 does not necessarily mean a net reduction of  $\text{CO}_2$  has occurred. It may be due to the existence of a reversible reaction involving  $\text{CO}_2$  as an end-product. At the present time no such reversible reactions in the respiratory process are known. It seems reasonable in view of the work of Hes and others that the formation of reduced radiocarbon by a respiring cell is due not to a simple interchange but rather that  $\text{CO}_2$  plays the rôle of a highly specific oxidizing agent.

It would seem that the entrance of a  $\text{CO}_2$  molecule into the system is necessary for the production of an additional large number of  $\text{CO}_2$  molecules. In a sense, then, respiration is auto-catalytic.

It has been most convenient to work with yeast and the results described below have been obtained with fresh yeast cells suspended in distilled water. The suspensions were exposed to a  $\text{C}^*\text{O}_2$  (0.5 to 2 cm.)-air mixture at approximately atmospheric pressure. Since short lived  $\text{C}^{11}$  (21-minute half-life) was used the exposures were of short duration (5 to 100 minutes). At various intervals  $\text{NaHCO}_3$  was added to the suspensions, which were then boiled vigorously with strong acid to remove dissolved  $\text{C}^*\text{O}_2$ . The method of measuring the activity has been described elsewhere.<sup>4</sup> The uptake of  $\text{C}^*\text{O}_2$  by aqueous suspensions of yeast cells as a function of length of time of exposure to  $\text{C}^*\text{O}_2$  is shown in figure 1.

The measurements are, of course, corrected for radioactive decay, and are therefore comparable.  $10^7$  counts/min. corresponds to 0.01 cc.  $\text{C}^*\text{O}_2$  (S. T. P.). The rate of  $\text{CO}_2$  production by each suspension was 0.01 cc. (S. T. P.)  $\text{CO}_2$  per minute. Thus under these conditions one  $\text{C}^*\text{O}_2$  molecule was reduced for every 50 molecules produced in respiration. These figures are to be considered merely semi-quantitative. A constant rate of  $\text{C}^*\text{O}_2$  reduction is obtained only when the amount of  $\text{C}^*\text{O}_2$  present at the start of the experiment is large compared to the  $\text{CO}_2$  evolved during the exposure. If this is not the case then the  $\text{C}^*\text{O}_2$  is diluted, and in addition lack of rapid equilibration between the freshly produced  $\text{CO}_2$  within the cell and the  $\text{C}^*\text{O}_2$  in the rest of the vessel becomes important. Thus at high respiratory rates the accumulation of reduced radioactive carbon may be less.

Attempts to chemically identify the active molecules have thus far been unsuccessful. When the cells are boiled with dilute acid for approximately

one minute the cell-free aqueous extract contains more than 90% of the reduced C\*. If the boiling acid treatment is omitted the cell-free medium has only ~1% of the activity. Osazones prepared with phenyl hydrazine as well as hydrazones of 2,4 dinitro phenylhydrazine are inactive (<1% of the activity). In the chemical analyses a mixture of aldehydes, sugars, acids, etc., was added to furnish carriers. Special attention was centered

$$\begin{array}{c} \text{O} \quad \text{O} \\ || \quad || \end{array}$$

on pyruvic acid since the decarboxylation of pyruvic acid ( $\text{CH}_3\text{C}-\text{C}-\text{OH} = \text{CO}_2 + \text{CH}_3\text{CHO}$ ) is known to play an important part in catabolic reactions. That the pyruvic acid fraction was inactive (<0.5%) indicates this reaction is irreversible. Salts of  $\text{Ba}^{++}$  precipitated in 80% ethanol were very active and even after several reprecipitations contained ~50% of the radioactivity. Decarboxylation of the active Ba salts was attempted at 250°C. for one hour. Only ~3% of the C\* was converted to  $\text{BaCO}_3$  by this treatment.

It is of interest at this point to mention that a non-photochemical<sup>5</sup> reduction of  $\text{CO}_2$  is carried out by green plants (barley, wheat, sunflower, chlorella, etc.). This seems to be definitely a part of the photosynthetic mechanism.<sup>6</sup> The dark pick-up of  $\text{CO}_2$  by plants has also been observed and measured by other investigators using different methods.<sup>7, 8</sup>

The evidence accumulated thus far indicates differences exist between the dark C\* $\text{O}_2$  reduction by a photosynthetic (chlorella) system on one hand and a non-photosynthetic (yeast) on the other. The time course of the C\* $\text{O}_2$  dark reduction by chlorella is shown in figure 2 and is to be contrasted with figure 1.

Decarboxylation experiments (dry distillation of the Ba salt at 250° for 1 hour) on the active material from chlorella suggest the major part of the C\* is present in a  $-\text{COOH}$  group. Furthermore the radioactive molecules formed in yeast were found in diffusion experiments to have a higher diffusion coefficient (and are very likely of lower molecular weight) than the active compounds formed in chlorella. The dark reduction of C\* $\text{O}_2$  by chlorella is reversible. It is not certain whether the C\* $\text{O}_2$  uptake by yeast is reversible. If the yeast cells are allowed to react with C\* $\text{O}_2$  for 60 minutes and then flushed with a continuous stream of  $\text{N}_2$  for 30 minutes, there is no decrease in the reduced C\* present in the cells. If a stream of (inactive)  $\text{CO}_2\text{-N}_2$  (50-50 mixture) is used, then ~15% of the reduced carbon is removed. Whether this loss occurs via the same path as the C\* $\text{O}_2$  reduction or by means of other reactions is still uncertain. In any case it is now an accepted fact that  $\text{CO}_2$  reduction is no longer an exclusive characteristic of photosynthetic and chemosynthetic autotrophic organisms.<sup>9</sup>

It is conceivable, as Professor G. N. Lewis and Professor W. C. Bray

have suggested to us, that the primary reactions resulting in  $\text{CO}_2$  reduction may be similar in many respects for photosynthetic and non-photosynthetic systems. The secondary reactions may differ enormously in the autotrophic as compared to the heterotrophic organisms, since in the former the net reaction is an accumulation whereas in the latter it is combustion of organic matter. It may prove easier to investigate the photosynthetic primary step because the secondary reactions take place only in the light. This is not the case in the heterotrophic systems since one must deal with a steady state rather than an equilibrium condition.

In conclusion, then, experiments with radioactive carbon have shown that a number of heterotrophic systems reduce small amounts of  $\text{C}^*\text{O}_2$ . The chemical identity of the active molecules is thus far unknown. These results offer positive evidence that  $\text{CO}_2$  is a specific oxidizing agent in

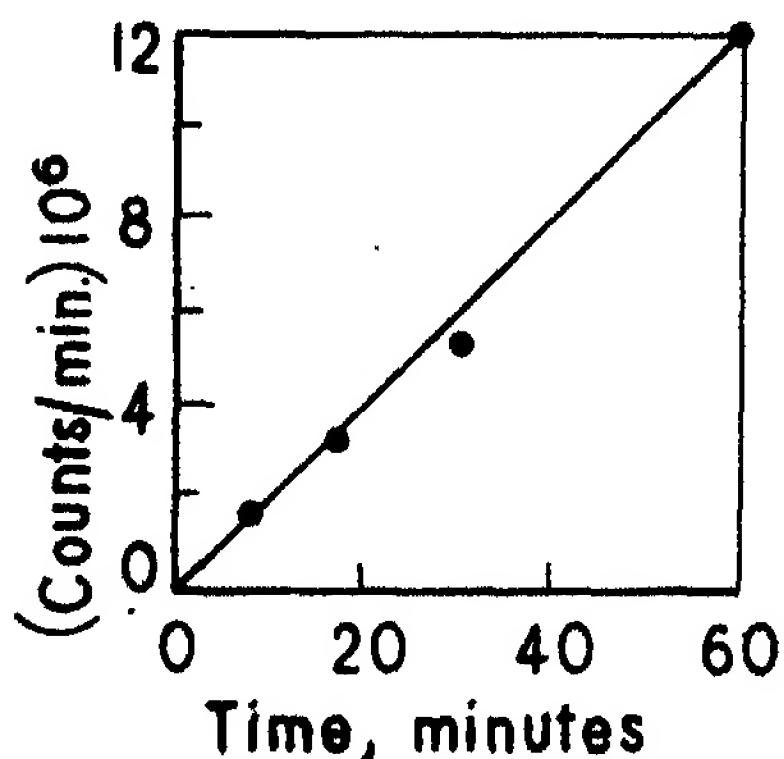


FIGURE 1

$\text{C}^*\text{O}_2$  assimilation by yeast.

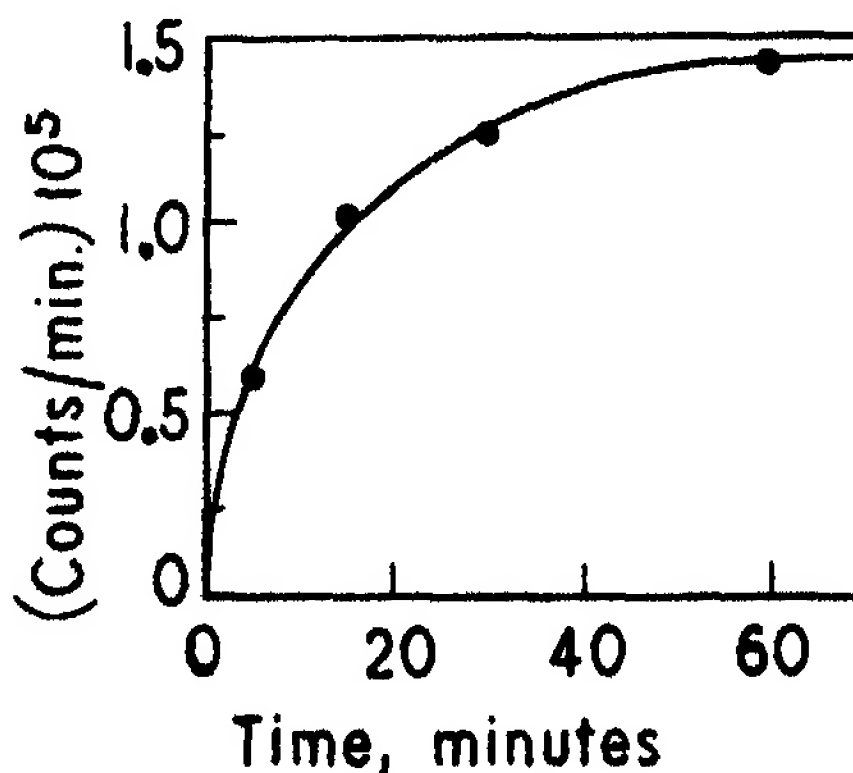


FIGURE 2

Dark  $\text{C}^*\text{O}_2$  reduction by chlorella.

respiratory processes. This suggestion was first proposed by several investigators as a possible explanation for the fact that small quantities of  $\text{CO}_2$  are essential for growth for microorganisms.<sup>1</sup>

It seems certain that further experiments with radiocarbon will yield important information regarding the mechanism.

We wish to thank Professor E. O. Lawrence for his interest and Dr. W. Z. Hassid for assistance in some of the experiments. We are indebted to Professor G. N. Lewis, Professor W. C. Bray and Professor H. A. Barker for helpful discussions and suggestions. Thanks are also due the Rockefeller Foundation for financial support to the Radiation Laboratory.

<sup>1</sup> For a summary, see Hes, *Ann. Fermentation*, 4, 547 (1938).

<sup>2</sup> Hes, *Nature*, 141, 647 (1938).

<sup>3</sup> We are indebted to Professor C. B. van Niel for the coli and Mr. A. R. Robinson for the preparation of the liver tissue.

- <sup>4</sup> Ruben, Hassid and Kamen, *Jour. Am. Chem. Soc.*, **61**, 661 (1939).  
<sup>5</sup> Ruben, Kamen, Hassid and DeVault, *Science*, **90**, 570 (1939).  
<sup>6</sup> Additional evidence to be published shortly.  
<sup>7</sup> McAlister, *Jour. Gen. Physiol.*, **22**, 613 (1939).  
<sup>8</sup> Emerson and Lewis, *Am. Jour. Botany*, **26**, 808 (1939).  
<sup>9</sup> Cf. van Niel, *Ann. Rev. Biochem.*, **6**, 606 (1937); Gaffron, *Ibid.*, **7**, 986 (1939).

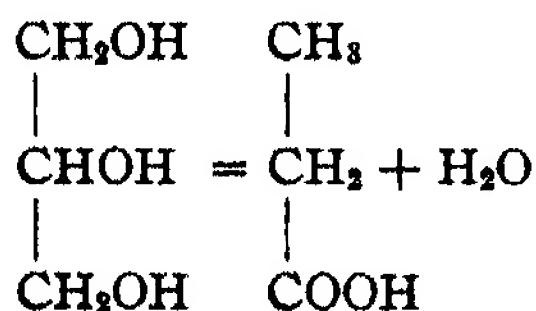
## CO<sub>2</sub> ASSIMILATION BY PROPIONIC ACID BACTERIA STUDIED BY THE USE OF RADIOACTIVE CARBON

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The studies of Wood and Werkman<sup>1, 2, 3</sup> as well as the work of Phelps, Johnson and Peterson<sup>4</sup> have shown that propionic acid bacteria can utilize CO<sub>2</sub> during the fermentation of glycerol. In the absence of CO<sub>2</sub> this fermentation can be adequately represented by the equation:<sup>5</sup>



In the presence of CO<sub>2</sub> the formation of propionic acid is accompanied by the appearance of succinic acid in amounts closely equimolar with the quantity of absorbed CO<sub>2</sub>.

This made it seem possible that CO<sub>2</sub> becomes converted into succinic acid by combination with a 3-carbon compound. The formation of succinic acid during the fermentation of pyruvate, dextrose and galactose by *Escher. coli*, particularly its dependence upon the CO<sub>2</sub> partial pressure,<sup>6</sup> supports this view.

It is apparent that important information regarding the mechanism through which CO<sub>2</sub> is utilized can be obtained by the use of radioactive CO<sub>2</sub>.<sup>7</sup> We have employed this approach in a study of the fermentation of glycerol by *Propionibacterium pentosaceum*.

The bacteria were grown anaerobically in yeast extract-glycerol media in the presence of CO<sub>2</sub> for 3 to 6 days. For the experiments cells from 250 to 500 ml. of such cultures were centrifuged, washed and suspended in 0.5 per cent phosphate buffer at pH 7.0 with and without added substrates. The suspensions were shaken at 30°C. in the presence of N<sub>2</sub> and C<sup>14</sup>O<sub>2</sub><sup>8</sup> for



30 to 40 minutes. After centrifuging off the cells, propionic acid and succinic acid were added to the supernatant liquid to provide carriers for the small quantities of radioactive metabolic products. The volatile and non-volatile acids were separated by exhaustive steam distillation; the cells were boiled gently with acid for a few minutes and separated from the acid extract by centrifugation. Special care was taken to remove all traces of dissolved  $C^*O_2$  before radioactivity measurements were made. The results of an experiment carried out in the presence of 2 per cent glycerol are summarized in table 1.

TABLE 1  
BACTERIA + 2 PER CENT GLYCEROL +  $C^*O_2$  FOR 40 MINUTES

	PER CENT OF TOTAL $C^*$ ASSIMILATED <sup>a</sup>
Volatile acids	72.0
Non-volatile acids	10.0
Boiled cell extract	17.5
Cells after extraction	0.5

<sup>a</sup> All measurements are corrected for decay and are therefore comparable.

The above table shows that 72 per cent of the  $C^*O_2$  taken up by the cells can be recovered in the volatile acid fraction and only 10 per cent in the non-volatile acid fraction. The boiled cell extract consisted mainly of acids carried down with the cells.

The activity in the volatile fraction was found to be restricted to propionic acid by a Duclaux distillation. The  $C^*$  content of successive distillates is compared in table 2 with the titration values obtained with pure propionic acid distilled under the same conditions.

PER CENT VOLUME OF DISTILLATE	PER CENT RADIOACTIVITY FOUND	PER CENT ACIDITY BY TITRATION FOR PURE PROPIONIC ACID
20.0	23.0	24.5
40.0	45.0	46.7
60.0	65.0	67.0
80.0	84.0	85.1
100.0	100.0	100.0

The agreement between the radioactivity and titration measurements is remarkably good. This result excludes the presence of more than 5% of  $C^*$  in other fatty acids (formic, acetic, etc.).

The radioactive component of the non-volatile acid fraction was identified as succinic acid in two ways. The distribution coefficients of the non-

volatile material between water and ethyl ether by titration and radioactivity measurements are compared in table 3.

TABLE 3  
DISTRIBUTION COEFFICIENTS  
(10 ml. Aqueous Phase + 90 ml. Ether)

	TITRATION	RADIOACTIVITY
K	7.4	6.0

In addition, the non-volatile material was sublimed and the specific activity  $\left(\frac{C^*}{\text{succinic acid}}\right)$  of the various fractions is shown in table 4.

TABLE 4  
SUBLIMATION

FRACTION	SPECIFIC ACTIVITY $\left(\frac{\text{RADIOACTIVITY}}{\text{SUCCINIC ACID}}\right)$
1 (210–220°C.)	9.5
2 (220–240°C.)	9.9

No radioactive material came off below 210°C. It seems quite reasonable to conclude that the radioactivity is mainly due to succinic acid.

The remarkable result that so much of the radioactive carbon is found in propionic acid might be explained by assuming a reversible reaction  $\text{CH}_3\text{CH}_2\text{COOH} + \text{CO}_2 \rightleftharpoons \text{COOHCH}_2\text{CH}_2\text{COOH}$ . Experiments were performed to test this hypothesis by the addition of propionic acid and succinic acids to cell suspensions in the presence of  $\text{C}^*\text{O}_2$ . In order to reduce the amount of glycerol and reserve products in the cell material, the bacteria were centrifuged from the medium in which they were grown, and suspended in phosphate buffer for 3 hours; this procedure was then repeated, and immediately before exposure to  $\text{C}^*\text{O}_2$  they were again washed. The results of this experiment are shown in table 5.

TABLE 5  
CELL SUSPENSION IN PHOSPHATE BUFFER EXPOSED TO  $\text{C}^*\text{O}_2$  FOR 30 MINUTES

SUBSTRATE	TOTAL $\text{C}^*$ ASSIMILATED (ARBITRARY UNITS)	RADIOACTIVITY OF VOLATILE ACID NON-VOL. ACID
Nothing	5.3	0.7
0.4 per cent Na propionate	4.8	0.78
0.2 per cent Na succinate	4.5	0.2
2.0 per cent glycerol	100.0	3.1

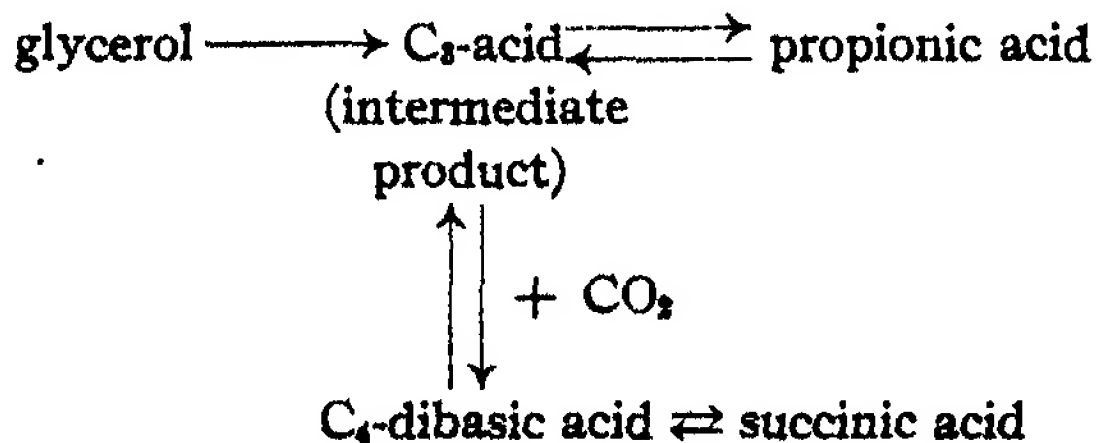
In a further experiment the cells were suspended in phosphate buffer for 7 hours and then centrifuged and washed as above. The results are summarized in table 6.

TABLE 6  
CELL SUSPENSION IN PHOSPHATE BUFFER EXPOSED TO C\*O<sub>2</sub> FOR 30 MINUTES

SUBSTRATE	TOTAL C* ASSIMILATED (ARBITRARY UNITS)	RADIOACTIVITY OF VOLATILE ACID NON-VOL. ACID
Nothing	2.3	0.41
2 per cent glycerol	100.0	3.0, 3.8
2 per cent glycerol + 0.4 per cent Na propionate	85.0	2.4

The addition of propionic acid in the absence of glycerol has little if any effect on the distribution of the C\* between the propionic and succinic acids or on the total C\* taken up. This is evidence against the formation of succinic acid from CO<sub>2</sub> and propionic acid. The presence of glycerol not only has an enormous effect on the C\*O<sub>2</sub> uptake, but also increases the ratio of radioactive carbon in propionic acid and succinic acid. It seems quite likely in view of these results that propionic and succinic acids are formed via reactions between CO<sub>2</sub> and glycerol or intermediate products arising during its fermentation. Pyruvic acid, which could be a possible intermediate, was found to be inactive (<0.5%).

The data so far presented could find an explanation on the basis of a mechanism such as:



In this case one would expect the radioactive carbon atom to be located in the carboxyl groups. Further experiments are in progress to test this hypothesis.

The results of the above experiments are of general interest also in connection with a major problem encountered in tracer experiments with labeled carbon, namely, the synthesis of radioactive molecules starting with C\*O<sub>2</sub>. In many cases synthetic organic methods result in poor yields despite time-consuming and painstaking effort. In such instances the use of appropriate microorganisms to perform the desired synthesis may be highly desirable. We may cite the above experiments as an example: In 30 minutes the bacteria converted over 80 per cent of the assimilated C\*O<sub>2</sub> into propionic and succinic acids, which are thus made readily available for tracer experiments.

It is a pleasure to thank Dr. M. D. Kamen and Professor E. O. Lawrence

of the Radiation Laboratory for their interest, and for making the radioactive carbon available. We are indebted to Professor C. B. van Niel and Professor H. A. Barker for much advice and assistance.

<sup>1</sup> *Biochem. Jour.*, **30**, 48 (1936).

<sup>2</sup> *Ibid.*, **32**, 1262 (1938).

<sup>3</sup> *Ibid.*, **34**, 7 (1940).

<sup>4</sup> *Biochem. J.*, **33**, 726 (1939).

<sup>5</sup> C. B. van Niel, *The Propionic Acid Bacteria*, Haarlem (1928).

<sup>6</sup> Elsdon, *Biochem. Jour.*, **32**, 187 (1938).

<sup>7</sup> Ruben, Hassid and Kamen, *Jour. Am. Chem. Soc.*, **61**, 661 (1939).

<sup>8</sup> The symbol C\* will be used to represent carbon labeled by the radioactivity of some of its atoms. The isotope is C<sup>11</sup> and was produced in the Berkeley cyclotron; it has a half-life of 21 minutes.

## THE REDUCTION OF RADIOACTIVE CARBON DIOXIDE BY METHANE-PRODUCING BACTERIA

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Communicated May 15, 1940

Several years ago<sup>1</sup> it was shown that the formation of methane in the fermentation of ethyl and butyl alcohols and of butyric acid by impure cultures of methane-producing bacteria is accompanied by the disappearance of an equi-molecular quantity of carbon dioxide. In the fermentation of ethyl alcohol, for example, the reaction can be rather closely described by the equation:



The same reaction has recently been shown<sup>2</sup> to be carried out by pure cultures of *Methanobacterium Omelianskii*.

As to the mechanism of the above reaction, it seemed quite likely that the acetic acid arises by an oxidation of ethyl alcohol, carbon dioxide being simultaneously reduced to methane. This would be in accordance with the view originally advanced by Professor C. B. van Niel that the methane fermentation of organic as well as of inorganic compounds is a process of oxidation by means of carbon dioxide which can be represented by the general equation:



where H<sub>2</sub>A is an oxidizable molecule and A is its oxidation product. Conclusive proof in favor of this mechanism, however, was lacking. Furthermore, no direct experimental evidence could be obtained in support of the

reduction of carbon dioxide in those very numerous methane fermentations of organic compounds which result in the formation of carbon dioxide. An example of such a process is the fermentation of methyl alcohol, the net reaction for which is



This reaction could—in accordance with the van Niel hypothesis—be the result of a complete oxidation of four molecules of methyl alcohol to carbon dioxide accompanied by a reduction of three molecules of carbon dioxide to methane. But such a reduction of carbon dioxide could obviously not be observed by gross chemical analysis.

Until recently there seemed no immediate hope that the applicability of the van Niel hypothesis to methane fermentations producing carbon dioxide could be tested. The production of intense samples of radioactive carbon in the Radiation Laboratory of the University of California<sup>3, 4</sup> has, however, made possible a direct and relatively simple test for carbon dioxide reduction even in such fermentations. For if radioactive carbon in the form of carbon dioxide is supplied to organisms fermenting organic compounds the methane produced must also be radioactive if the van Niel hypothesis is correct. If it is incorrect the methane will be inactive.

Radioactive carbon\* experiments have been carried out with pure cultures of two species of methane-producing bacteria, *Methanobacterium Omelianskii* and *Methanosarcina methanica*.<sup>†</sup> The former organism is able to oxidize simple primary and secondary alcohols only as far as the corresponding fatty acids and ketones, respectively, and is known from chemical evidence to reduce carbon dioxide in accordance with equation (2). The latter organism oxidizes methanol, acetic acid and probably other compounds completely to carbon dioxide, so preventing direct chemical observation of carbon dioxide utilization.

The experiments were carried out with heavy cell suspensions prepared by centrifuging bacteria from 1–2 liters of medium, washing and resuspending in 10–15 cc. of a phosphate buffer solution (pH 7.0) containing 0.01%  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ . The final suspensions contained 1.0–1.2 mg. of cell nitrogen or about 10–12 mg. dry weight of ash-free cells per cubic centimeter.

*Experiments with Mb. Omelianskii.*—The experiments with *Mb. Omelianskii* were undertaken with two objectives. The first was to obtain direct evidence concerning the reduction of carbon dioxide to methane. As has already been mentioned, the chemical evidence already very strongly indicated such a reaction. The second objective was to find out whether carbon dioxide is also converted into cell material. Carbon balance experiments described elsewhere<sup>2</sup> have shown that the synthesis of cell material in media containing ethanol and carbon dioxide occurs in one of two ways. Either (1) the cell material is derived exclusively from ethanol, carbon

dioxide being converted only into methane, or (2) the cell material is derived both from ethanol and carbon dioxide. A decision between these two possibilities can be reached by determining whether or not radioactive carbon dioxide gives rise to radioactive cell material.

For the experiments suspensions of bacteria grown in an ethanol-bicarbonate medium were used. The bicarbonate supply was adjusted so as to be limiting; at the end of the fermentation, the growth medium was free of carbon dioxide. Two experimental vessels were used, both of which contained 5 cc. of cell suspension plus 0.5 cc. of 8% (vol.) ethanol. To one vessel was added 0.5 cc. of a 10%  $\text{HgCl}_2$  solution. Both suspensions were incubated for 40 minutes at  $40^\circ\text{C}$ . after adding the radioactive carbon dioxide. At the end of the incubation period the gases were pumped out of the vessels and the residual carbon dioxide removed by absorption in alkali. The methane was then combusted by passing over hot copper oxide; the resulting carbon dioxide was absorbed in alkali. The radioactivity of the alkali carbonate solution was measured by means of a Geiger counter.

The cell suspension from each vessel was acidified to pH 1 with  $\text{H}_2\text{SO}_4$  and steam-distilled to separate the volatile acid. The residuum was concentrated to dryness on a hot plate to free the cells of carbon dioxide; the radioactivity was then measured. Control experiments showed that carbon dioxide is completely removed by the preliminary acid treatment. The radioactivities of the cells and methane are given in table 1. The volatile acid fraction was inactive. The data in table 1 are, of course, corrected for decay to a convenient time so that they are directly comparable.

TABLE 1

REDUCTION OF CARBON DIOXIDE BY *Mb. Omelianskii*

(Activities are expressed in counts per minute. Background = 30 counts per minute)

EXPERIMENT	TOTAL ACTIVITIES OF	
	METHANE	CELLS
Living cells	$12 \times 10^4$	$18 \times 10^4$
Dead cells ( $\text{HgCl}_2$ )	...	0

The formation of radioactive methane in this experiment shows conclusively that carbon dioxide is reduced to methane by *Mb. Omelianskii*. The quantity of radioactive methane formed was sufficient to give a count of the order of 4000 times greater than the background count. The absence of any radioactivity in the volatile acid fraction provides direct evidence against the view that methane is formed via acetic acid.

The results also show that carbon dioxide is converted in easily demonstrable amounts into cell material by living cells. Dead cells are completely inactive. In connection with other evidence,<sup>2</sup> it must therefore be concluded that both ethanol and carbon dioxide are used in the synthesis of



cell material. The ratio of radioactivities of cells and methane indicates that about 1.5% of the carbon dioxide reduced is so used.

*Experiments with Ms. methanica.*—This organism was grown in a medium containing 0.6% (vol.) of methanol. When the fermentation had almost stopped, the bacteria were centrifuged and resuspended as described above. The action of cell suspensions was tested upon methanol and sodium acetate; the technique of the experiments was the same as in those of *Mb. Omelianskii*. Since the experiments were essentially qualitative in nature, only the general results will be described.

The experiments showed quite conclusively that *Ms. methanica* also is able to reduce carbon dioxide to methane. Radioactive methane was obtained from suspensions provided with methanol or with sodium acetate. However, since the quantity of radioactive gas was of the same order of magnitude in both cases and since the presence of small amounts of methanol (from the growth medium) in the acetate experiment could not be completely prevented, the evidence for the reduction of carbon dioxide to methane as a result of the oxidation of acetic acid remains inconclusive. The reduction of carbon dioxide to methane as a result of the fermentation of methanol can be considered definitely established.

Although in the fermentation of methanol, carbon dioxide is reduced to methane, it should be emphasized that the data obtained are not adequate to show whether or not *all* the methane originates in this way. It is also possible that the alcohol might be directly reduced to methane. Further experiments are required to elucidate this point.

It has been found that *Ms. methanica* also utilizes carbon dioxide in the synthesis of cell material. The ratio of radioactivity of cells to methane is about 1:10. From this ratio and the equation for the fermentation of methanol, it can be estimated that the quantity of carbon dioxide assimilated is sufficient to account for at least a large part of the cell synthesis. The exact quantity of cell substance formed by this organism is not known, but anaerobic bacteria generally and *Mb. Omelianskii* in particular are known to assimilate only 5–10% of the total substrate decomposed.

*Conclusions.*—Although the above experiments deal with only two of the many kinds of methane-producing bacteria and with only three of the many organic compounds fermented by these organisms, they considerably strengthen the generalization that the methane fermentation of organic as well as of inorganic compounds is essentially an anaerobic oxidation process in which carbon dioxide acts as the ultimate hydrogen acceptor (oxidizing agent) and is reduced to methane. And it has been established that methane bacteria, like many other living organisms,<sup>†</sup> have the ability to convert carbon dioxide into organic constituents of their cells.

This work was made possible by the invaluable coöperation of Professor E. O. Lawrence and members of the Radiation Laboratory. Financial aid

from the Rockefeller Foundation to the Radiation Laboratory is gratefully acknowledged.

Clerical assistance was furnished by the personnel of the Works Progress Administration official Project No. 65-1-08-91-B-10.

\*  $C^{11}$  (21-minute half-life) was used in our experiments.

† The former organism was isolated by one of us (H. A. B.). The latter was isolated by Mr. Schnellen (Delft) and was obtained through the courtesy of Professor A. J. Kluyver. To both Mr. Schnellen and Professor Kluyver the authors wish to express their appreciation.

‡ Unpublished experiments by Ruben, Hassid and Kamen have shown that many types of heterotrophic organisms and tissues including yeast, *B. coli*, propionic acid bacteria, liver (rat), algae and various parts of higher plants can reduce carbon dioxide in the dark. It seems quite likely that this is a general property of living organisms. See *Proc. Nat. Acad. Sci.*, **26**, 418-422 (1940).

<sup>1</sup> Barker, H. A., *Arch. Mikrobiol.*, **7**, 404-419 (1936).

<sup>2</sup> Barker, H. A., Unpublished.

<sup>3</sup> Ruben, S., Hassid, W. Z., and Kamen, M. D., *Jour. Am. Chem. Soc.*, **61**, 661-663 (1939).

<sup>4</sup> Ruben, S., Kamen, M. D., Hassid, W. Z., and DeVault, D. C., *Science*, **90**, 570-571 (1939).

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## THE CORONAVISER, AN INSTRUMENT FOR OBSERVING THE SOLAR CORONA IN FULL SUNLIGHT

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The old problem of attempting to observe the solar corona in full sunlight is not only of interest to astronomers but also to those radio engineers who are concerned with radio transmission over long distances. The major disturbances of such transmission have their origin in the sun and studies to date have indicated that a day to day knowledge of the activity of the corona might prove useful in predicting the transmission conditions.

The recent success of Lyot<sup>1</sup> leaves much to be desired in the way of a continuous record of coronal activity. A method of greater discrimination is needed. Such a method was proposed<sup>2</sup> several years ago and is based on the use of television technique, the corona being separated from the glare by electrical filters while the image of the sky around the sun is in the form of an electric current.

This is a report of the first practical trial of the method. Special television apparatus has been developed at the Bell Telephone Laboratories for use in conjunction with the 15" horizontal refractor of the Cook Observatory<sup>3</sup> at Wynnewood, Pa. See figure 1. The input scanner is me-

chanical and scans a ring-shaped area in the sky around the sun, the flying spot tracing out the area along a spiral path. This scanner is shown in figure 2. The back-silvered lens  $L$  by rocking while it rotates effectively scans the sky image in the plane of the masking disc  $D$  with the scanning hole  $H$ . The apparatus at the other end of the motor shaft generates the

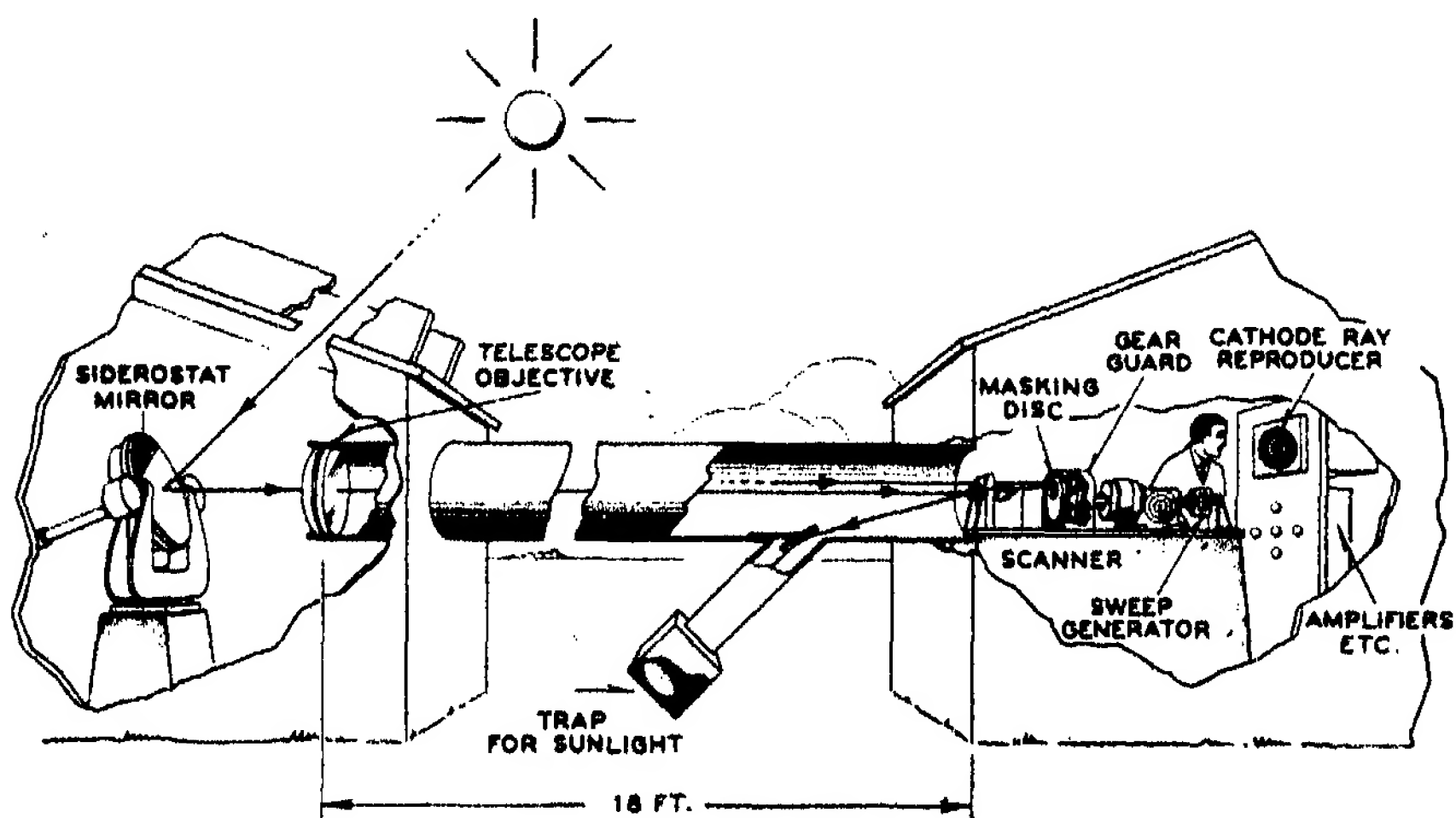


FIGURE 1

Layout of the apparatus at the Cook Observatory.

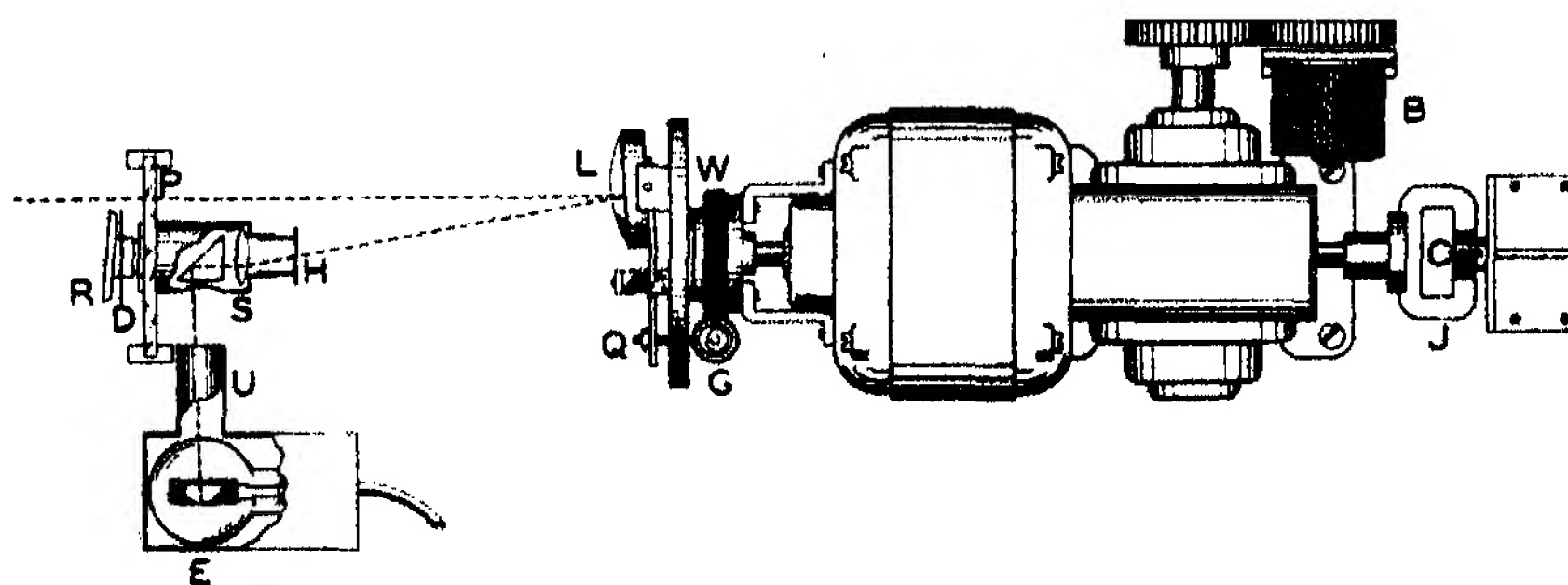


FIGURE 2

The input scanner and sweep voltage generator.

electrical waves which direct the electron beam of the reproducing cathode-ray tube around a path corresponding with that of the input. A detailed description of the technical details of all of the apparatus is published elsewhere.<sup>4</sup>

The glare of the clear sky is uniform angularly around the solar image and, therefore, as the scanning spot travels around the field, it gives rise

mainly to a direct current in the photocell. But the coronal features, that is, the streamers, arches, etc., generate a current of varying amplitude which amounts to an alternating component in the photoelectric current and only this latter component is amplified so that the reproduction contains, ideally at least, only the coronal image. Inaccuracies of alignment and nonuniformity of the intensity of the glare across the field occasioned by instrumental defects give rise to fairly strong low frequency components, particularly at the circular scan frequency or fundamental of the image signal. A high-pass filter whose cut-off characteristic could be altered was included in the circuit in order to eliminate such undesired signals. There was also included a low-pass filter to eliminate high frequency noise. The photocell (*E*, Fig. 2) had a caesium sulphide surface with a spectral characteristic that is a fairly close match to that of sunlight.

Since the inner corona has a surface brightness of about the same magnitude as the full moon, the sensitivity of the apparatus was checked by obtaining images of the moon in its various phases. These intensity levels are of the order of a millionth of that of the brightness of the solar surface. The magnitude of the glare through which it was necessary to work was of the order of 1000 times these values. This high level of glare was probably due to scattering by telescopic parts, particularly the siderostat mirror, rather than the sky. There were, however, many days when the glare from the sky had a magnitude considerably higher than this value.

Extreme care had to be taken to keep the optical parts of the apparatus clean. The slightest speck of dust on certain surfaces of the optical parts gave rise to overloaded images on the cathode ray tube, for the essence of the method is the amplification of minute variations in the intensity of illumination from point to point in the field. Occasionally tiny specks of brilliant light floated across the screen. These were finally traced to wind-borne seeds and insects which drifted across the sky between the apparatus and the sun.

It was necessary to have an absolute criterion by which one could distinguish between parasitic images which were caused by instrumental defects and coronal and prominence images which were associated with the sun. Such a test was furnished by the horizontal mounting of the telescope. By virtue of this arrangement, the celestial field rotates about the optic axis of the telescope with time. Photographs were taken at successive intervals over a period of several hours during which time the celestial images rotated through a sufficient angle (about  $7^\circ$  per hour) to distinguish them from the stationary parasitic images.

A large number of prominences have been observed and photographed with the apparatus. Figure 3 shows the prominences around the sun on October 31, 1938. This was taken with a red glass filter (Schott RG2)

FIGURE 4  
A jet or flare in the corona  
photographed on October 18,  
1938.

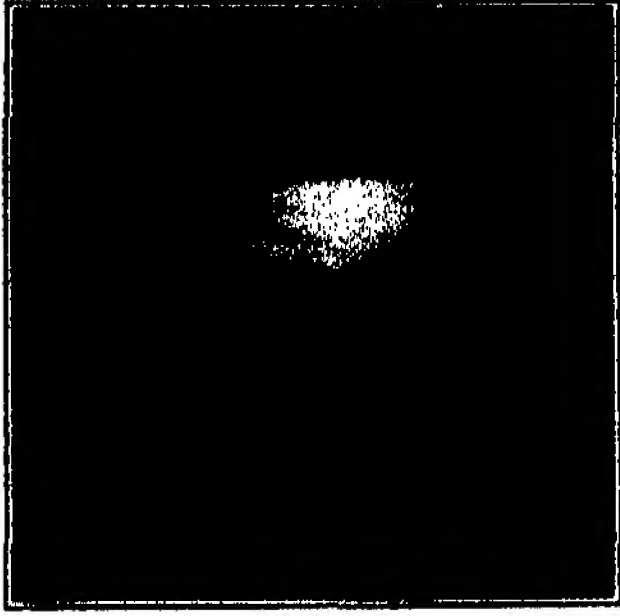
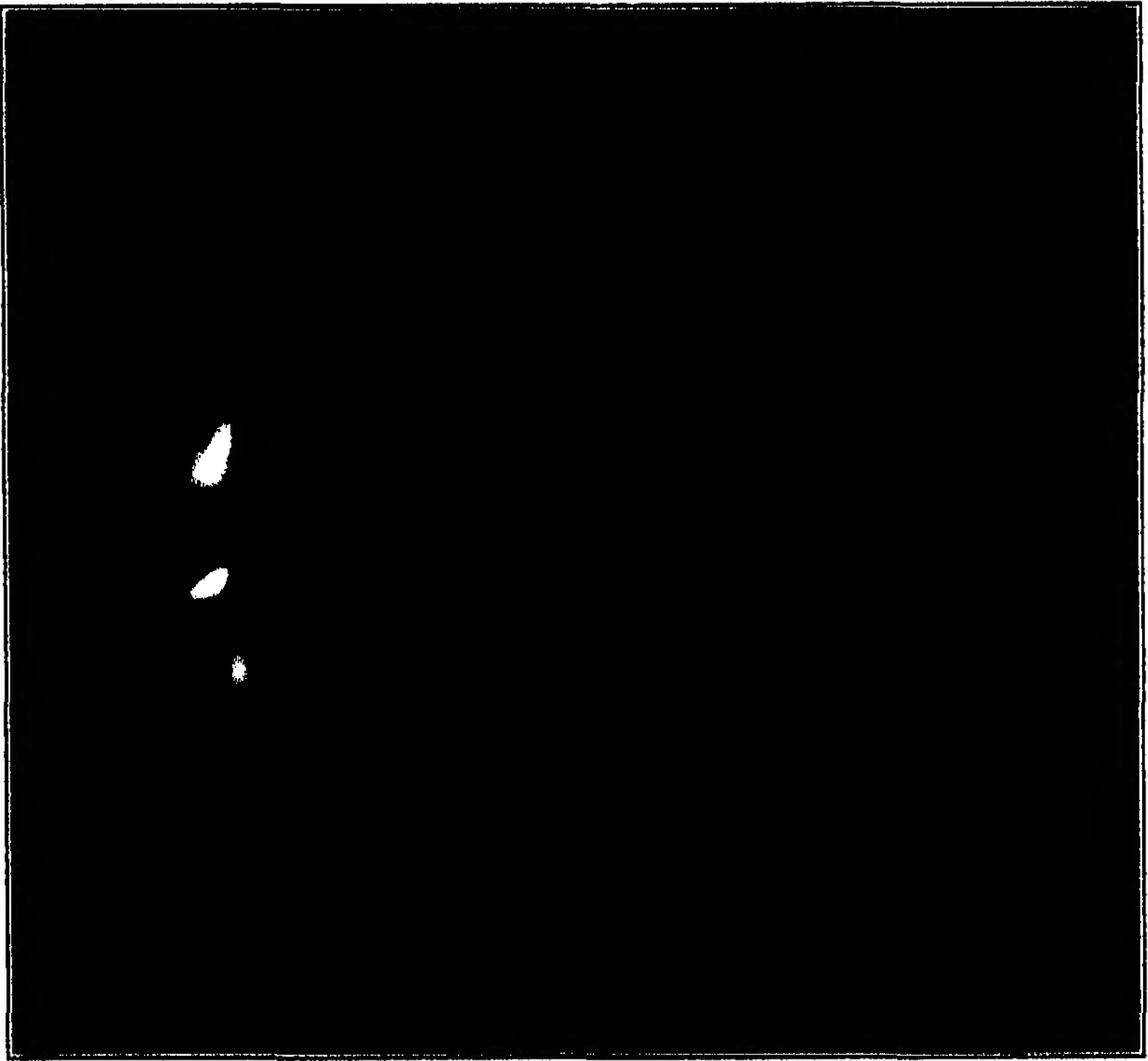


FIGURE 3  
Prominences around the sun on Oct. 31, 1938.







in front of the photoelectric cell. Good images were also obtained of prominences without any optical filter, i.e., in white light.

A number of the images showed features which apparently belong to the corona. Figure 4 shows such an image, it being identified by the test mentioned above as a flare or jet in the corona. This image was taken without any optical filter. It is one of 11 photographs that were taken over a period of more than two hours.

The major objectives of this phase of the work which were the development of an adequate instrument and the proving in of the method, have been achieved. The next phase of the investigation will be carried out under the more favorable conditions at the McDonald Observatory in Texas under the direction of Dr. Otto Struve.

<sup>1</sup> Lyot, B., *M. N. R. A. S.*, 99, No. 8, 580 (1939).

<sup>2</sup> Skellett, A. M., *Proc. Nat. Acad. Sci.*, 20, 461 (1934).

<sup>3</sup> The author is indebted to Dr. Cook and his associates for their able coöperation.

<sup>4</sup> *Bell System Tech. Jour.*, 19, 2, 249 (1940).

## A PATHOLOGICAL CASE IN THE NUMERICAL SOLUTION OF INTEGRAL EQUATIONS

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Communicated April 17, 1940

Consider an integral equation of the first kind

$$f(u) = \int_0^{\infty} H(v)K(v, u)dv \quad (1)$$

where  $f(u)$  and the kernel  $K(v, u)$  are given, and  $H(v)$  is to be determined from equation (1). Such equations are of frequent occurrence in physical problems,<sup>1</sup> where it often happens<sup>2</sup> that the analytical form of the kernel is known, but that  $f(u)$  may be a function determined observationally at only a discrete set of values of  $u$ .  $f(u)$  is then known only to within the errors of the observations and the question arises as to what accuracy  $H(v)$  can be determined from equation (1). It is clear that  $H(v)$  is subject to at least the uncertainties of  $f(u)$ . An additional uncertainty will arise from the approximate nature of the method of solution of the integral equation, whether this method be numerical or mechanical, and it is this type of uncertainty which will be discussed in the present note. One might be tempted to expect that, if a trial solution  $H_1(v)$  when inserted in the right-hand side of equation (1) yields an  $f_1(u)$  which differs from  $f(u)$  by only a

few per cent,  $H(v)$  is approximated to by  $H_1(v)$  to the same degree of accuracy. The case to be cited presently shows that generally such an expectation is altogether unjustified. The integral equation is

$$f(u) = \frac{\sqrt{u + 1/3}}{(2u + 1)^2 - 4u\sqrt{u + 1/3}\sqrt{u + 1}} = \int_0^\infty \frac{H(v)dv}{(u + v)^{1/2}} \quad (2)$$

For small values of  $u$

$$f(u) = \frac{1}{\sqrt{3}} - \left(\frac{5}{2\sqrt{3}} - \frac{4}{3}\right)u - \dots, \quad (3)$$

and for large values of  $u$

$$f(u) \rightarrow \frac{3}{4\sqrt{u}} - \frac{9}{16u^{3/2}} + \dots \quad (4)$$

For real values of  $u$ ,  $f(u)$  is a well-behaved function, as is shown in the second column of table 1. Equation (2) arises in the seismological problem of the motion of the surface of a uniform elastic half-space due to the sudden application of a localized normal stress  $p_{zz}$  at the surface. The variation with time of  $p_{zz}$  is represented by the Heaviside unit function  $H(t)$  and its space localization is such that it is everywhere zero, except at the origin of coördinates where it becomes infinite in such a manner that

$$2\pi \int_0^\infty p_{zz}(r)rdr = Z, \quad (5)$$

$Z$  being a (negative) constant. It is further assumed that Poisson's ratio is  $1/4$ , so that the velocity of the dilatational wave is  $\sqrt{3}c$ , where  $c$  is the velocity of the shear wave. This problem was originally solved by Lamb<sup>3</sup> by Fourier methods and the solution to be given presently for a pulse of the form  $H(t)$  can be derived from his results. Lamb's method of solution is, however, very cumbersome and the author found that the solution of this problem, as well as of the more difficult problem for a layered half-space, can be obtained more expeditiously by operational methods. The details of the solution will be presented elsewhere and we shall give here only the results for the vertical component of motion  $w$ . Let  $r$  denote the distance on the surface from the origin,  $\mu$  the coefficient of rigidity and

$$v = (ct/r)^2, w(t, r) = - (Z/\pi\mu r)H(v).$$

Then

$$H(v) = \begin{cases} 0, & v < 1/3, \\ \frac{1}{16} - \frac{\sqrt{3}}{32\sqrt{v - 1/4}} + \frac{\sqrt{3\sqrt{3} - 5}}{32\sqrt{v - 1/4}(3 - \sqrt{3})} - \frac{\sqrt{3\sqrt{3} + 5}}{32\sqrt{1/4}(3 + \sqrt{3}) - v}, & 1/3 < v < 1, \end{cases}$$

$$H(v) = \frac{2}{3} - \frac{\sqrt{3\sqrt{3} + 5}}{16\sqrt{\frac{1}{4}(3 + \sqrt{3}) - v}}, \quad 1 < v < \frac{1}{4}(3 + \sqrt{3}),$$

$$H(v) = \frac{2}{3}, \quad v > \frac{1}{4}(3 + \sqrt{3}). \quad (6)$$

It can be verified by substitution in equation (2) that equation (6) is an exact solution. This solution is plotted as curve *A* in figure 1. It is seen

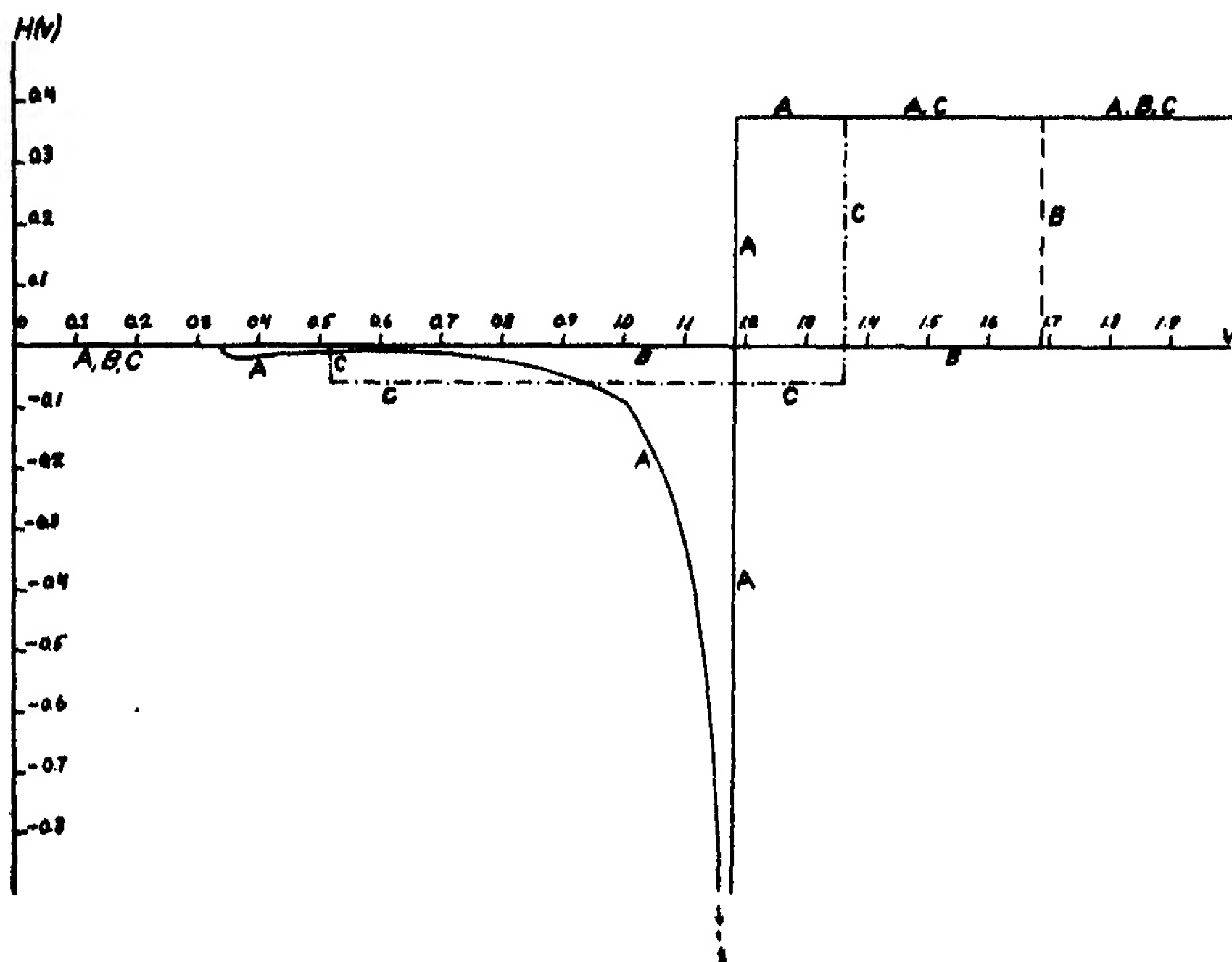


FIGURE 1

Curves *A*, *B*, *C* represent  $H(v)$ ,  $H_1(v)$  and  $H_2(v)$ , respectively. The associated  $f$ 's are given by equations (2), (8) and (10).

that there is no motion until the arrival of the *P* wave at  $v = \frac{1}{3}$ ; the shear wave, which is due to arrive at  $v = 1$ , is marked only by a discontinuity in slope. At  $v_0 = 1.183$  the Rayleigh surface wave arrives and the amplitude becomes infinite as  $(v_0 - v)^{-1/2}$ . If  $t_0$  is the arrival time of the Rayleigh wave, then for  $t = t_0 - \delta$ , where  $\delta$  is small,  $H(v)$  is proportional to  $\sqrt{r/\delta}$ . The infinity in  $w$  associated with the Rayleigh wave is therefore proportional to  $r^{-1/2}$ , which is characteristic of surface waves. Beyond  $v_0$  the steady-state displacement is immediately obtained. From a seismological point of view, the chief interest lies in the nature of the discontinuities that are associated with the arrivals of *P*, *S* and Rayleigh waves, because the

recognition of these phases enables one to determine the velocities of medium.

Now returning to the integral equation (2) we see that a trial function  $H_1(v)$  which is zero up to  $v = a$  and is equal to  $A$  for  $v > a$ , yields an

$$f_1(u) = \frac{2A}{\sqrt{u+a}}. \quad (7)$$

Let us try a solution of this form and determine the constants  $A$  and  $a$  so that  $f_1(u)$  will agree with  $f(u)$  in the leading terms of the expansions (3) and (4). This yields

$$f_1(u) = \frac{3}{\sqrt{16u+27}} \quad (8)$$

and the corresponding  $H_1(v)$  is plotted as curve  $B$  in figure 1. As a seismogram, curve  $B$  is totally inadequate and yet, as is seen from the third column of table 1, the maximum deviation of  $f_1(u)$  from  $f(u)$  is only of the order of 2%. The situation is even more aggravated by the trial solution  $H_2(v)$  which is given by

$$\begin{aligned} H_2(v) &= 0, v < 0.517787483 \\ H_2(v) &= -0.0612441866, 0.517787483 < v < 1.362107488, \\ H_2(v) &= 3/8, v > 1.362107488. \end{aligned} \quad (9)$$

This function is plotted as curve  $C$  in figure 1. It was chosen so that the expansions of

$$f_2(u) = -\frac{0.122488373}{\sqrt{u+0.517787483}} + \frac{0.872488373}{\sqrt{u+1.362107488}} \quad (10)$$

agree with the four terms in the expansions of  $f(u)$  which are given in equations (3) and (4). Curve  $C$  is again a bad sample of a seismogram for the medium in question. It yields a velocity of the  $P$  wave which is wrong by 25%; there is no sign of an  $S$  wave, and the phase corresponding to the Rayleigh wave arrives too late by 7%. In practice this phase would easily be mistaken for an  $S$  phase, because its amplitude diminishes with distance like  $r^{-1}$  instead of the  $r^{-1/2}$  which is characteristic of Rayleigh waves, and this mistaken  $S$  wave would yield a shear velocity which is wrong by 17%. In spite of these deficiencies,  $f_2(u)$  is seen from table 1 to differ from  $f(u)$  by less than 0.1%.

TABLE 1

$u$	$f(u)$	$\frac{f_1(u)}{f(u)}$	$\frac{f_2(u)}{f(u)}$
0	0.5773503	1.000	1.000000
0.1	0.5656129	0.992	1.000184
0.4	0.5289999	0.981	1.000780
0.5	0.5175205	0.980	1.000857
1.0	0.4678638	0.978	1.000857
2.0	0.3984167	0.980	1.000555
3.0	0.3523105	0.983	1.000362
4.0	0.3190756	0.986	1.000251
6.0	0.2735410	0.989	1.000139

*Summary.*—An integral equation of the first kind arising in a seismological problem is cited for which a trial solution which is totally unacceptable reproduces the given function on the left-hand side to within 2%. A second trial solution, which is physically wrong by 25% and is otherwise deficient, reproduces the given function to within 0.1%.

<sup>1</sup> A good treatment of integral equations from the point of view of their applications will be found in Hamel, G., *Integralgleichungen*, Berlin, Springer (1937).

<sup>2</sup> Pekeris, C. L., *Gerl. Beil. z. Geoph.*, 41, 192–202 (1934).

<sup>3</sup> Lamb, H., *Phil. Trans. Roy. Soc.*, (A), 203, 1 (1904).

## THE CONFORMAL THEORY OF CURVES

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In this note, we announce some of the principal results of a new conformal theory of curves in a Riemann space<sup>1</sup>  $V_n$ . The proofs of these results as well as the detailed conformal geometry of curves will be given elsewhere. In general, we assume that  $V_n$  is a Riemann space of class  $C^2$ . The precise hypotheses concerning existence, continuity and differentiability will appear in the paper which contains the proofs of these theorems.

Since every analytic curve in a  $V_2$  is conformally equivalent to a straight line in the plane, there can be no conformal theory<sup>2</sup> of (single) curves in a  $V_n$  unless  $n > 2$ . We consider those properties of a curve which remain unchanged when the enveloping Riemann space  $V_n$  of dimensionality  $n > 2$  undergoes any conformal mapping, not necessarily on itself. While the results obtained bear a close analogy to those which hold in the metric theory, in some cases the proofs are markedly different.

The principal tool is a new kind of tensor differentiation which has conformal meaning. This *conformal derivative* is dependent not only on

the metric of  $V_n$  but also on the curve  $C$  in  $V_n$  with respect to which the differentiation takes place. This dependence of conformal differentiation on a curve as well as the space is analogous to the similar dependence of parallel displacement of vectors in a general Riemann space. We find  $n - 1$  differential conformal curvatures  $J_1, J_2, \dots, J_{n-1}$  and an integral conformal arc length  $S$  which are unchanged by any conformal transformation of  $V_n$ . This means that

**THEOREM 1.** *If  $V_n \longleftrightarrow \bar{V}_n$ ,  $C \longleftrightarrow \bar{C}$  by a conformal map, then a conformal arc length parameter  $S$  may be chosen so that the corresponding points of  $C$  and  $\bar{C}$  have the same value of  $S$  and  $J_1, J_2, \dots, J_{n-1}$  are the same functions of  $S$  for  $C$  and  $\bar{C}$ .*

We prove the existence theorem

**THEOREM 2.** *In any  $V_n$ , a curve exists whose conformal curvatures are any arbitrary continuous functions of the conformal arc length.*

This curve is uniquely determined by a set of initial conditions which is found explicitly. In spaces conformal to a euclidean space, the following fundamental conformal equivalence theorem for curves exists which is the analogue of the (metric) congruence theorem:

**THEOREM 3.** *Let  $C_1$  and  $C_2$  be curves in the conformally euclidean spaces  ${}_{(1)}\bar{R}_n$  and  ${}_{(2)}\bar{R}_n$ , respectively, whose conformal curvatures are the same functions of the conformal arc length. Then a conformal transformation exists so that  ${}_{(1)}\bar{R}_n \longleftrightarrow {}_{(2)}\bar{R}_n$  and  $C_1 \longleftrightarrow C_2$ .*

The conformal curvatures have rather simple geometric properties if  $V_n$  is conformal to an Einstein space or, more particularly, to a euclidean space. For example, we mention the following theorems:

**THEOREM 4.** *The most general conformal differential invariant of a curve in an  $\bar{R}_n$  is a function of the conformal curvatures and their derivatives with respect to a conformal arc length parameter. Conversely, every such function is a conformal differential invariant.*

Examples may be given to show that this theorem is untrue in spaces which are not conformally euclidean.

**THEOREM 5.** *The necessary and sufficient condition that  $J_\alpha \equiv 0$  ( $0 < \alpha < n - 1$ ) along a curve  $C$  in an  $\bar{R}_n$  is that  $C$  be conformally equivalent to a curve in  $R_n$  whose  $(\alpha + 1)$ st metric curvature is identically zero.*

As an important special case, this theory includes the "natural geometry of curves" (according to the viewpoint of Cesàro, Pick and Kowalewski) in  $R_n$  under the continuous group of conformal mappings of  $R_n$  on itself. In particular, the curves along which each conformal curvature is equal to a constant are the paths of the group.

If  $n = 2$ , the above theory applies if the conformal mappings are restricted to transformations (applied to surfaces of constant curvature) which are similar to and include the inversive transformations of the plane. In this case, the present theory includes the inversive theory of



plane curves developed by Mullins, Liebmman, Kubota, Morley and Patterson who used different methods than those employed here.

The methods and results of this note may also be used to develop a theory of curves in a conformal Riemann space. From this point of view, this theory is related to the work of Hlavatý, Sasaki, Yano and others on curves in a conformal Riemann space.

A preliminary investigation tends to show that a conformal theory similar to that outlined above exists for any subspace in  $V_n$ . At points of a subspace, it is possible to define a "conformal derivative" and to arrive at a sequence of normal vector spaces and fundamental forms which are unchanged by conformal transformations of  $V_n$ .

<sup>1</sup> We denote an  $n$ -dimensional Riemann space and euclidean space by  $V_n$  and  $R_n$ , respectively. Spaces conformal to  $V_n$  and  $R_n$  are denoted by  $\bar{V}_n$  and  $\bar{R}_n$ , respectively.

<sup>2</sup> The general conformal geometry for two dimensions (equivalent to the theory of analytic functions of a complex variable), developed by Kasner since 1910, deals with invariants, absolute and relative, of two or more curves (horn angles and trihornometry). See *Trans. Amer. Math. Soc.*, **44**, 25-31 (1938), and these PROCEEDINGS, **23**, 337-341 (1937).



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TRAUMATIC ACID AND THIAMIN AS GROWTH FACTORS FOR  
ALGAE<sup>1</sup>

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Communicated June 7, 1940

Bottomley and, especially, Ashby<sup>2</sup> showed that *Lemna* develops well in a purely inorganic medium, although it thrives better if some organic matter, such as a soil extract, is added. The addition of soil extract and other chemically ill-defined organic matters<sup>3</sup> to the culture medium of algae is a general practice.<sup>4</sup> Since the chemistry of many plant hormones has become known in recent years it seemed of interest to see in which respect they affect the growth of algae. Yin<sup>5</sup> studied the effect of auxin on *Chlorella* and found an inhibition of the multiplication but an increase of the individual cell size. Pratt,<sup>6</sup> however, found just the reverse, an increase in cell number and no increase in cell size. Recently English, Bonner and Haagen-Smit<sup>7</sup> isolated and synthesized traumatic acid (1-decene-1, 10 dicarboxylic acid), a wound hormone of plants, which causes considerable proliferation and elongation of parenchymatous tissues (bean pods and potato tubers). On the assumption that this substance might exhibit similar properties on plant cells in general, it was added to the sterile cultures of unicellular algae<sup>8</sup> in order to determine whether it promotes the growth. Traumatic acid indeed proved to be highly effective in promoting multiplication of the culture, as is shown in the table for *Scenedesmus obliquus*, *Sc. bijugatus*, *Sc. brasiliensis* and *Palmellococcus miniatus*. Similar results were obtained with *Coccomyxa subellipsoidea* forma *putridena*, *Kirchneriella spec.?*, *Coelastrum proboscideum* var. *gracilis* and *Stichococcus bacillaris*.<sup>9</sup>

Thiamin was recognized as a growth factor for fungi by Schopfer<sup>10</sup> and later on also as a growth factor for higher plants.<sup>11</sup> Thiamin was found to be ineffective, or at least far less effective than traumatic acid, when added to cultures (in basic inorganic medium) of the previously mentioned algae. It proved, however, to be decidedly effective in promoting the growth of *Sphaerella lacustris* (*Haematococcus pluvialis*).<sup>12</sup> Traumatic acid also af-

fects the growth of *Sphaerella lacustris* but to a lesser extent than vitamin B<sub>1</sub>.

Adenine has lately been shown to be active as a factor for leaf growth.<sup>13</sup> No algae have been found so far that respond markedly to it.

The above-mentioned experiments show that both traumatic acid and thiamin are growth factors for algae.

MULTIPLICATION OF ALGAE UNDER STERILE CULTURE CONDITIONS, AS MEASURED BY PHOTOELECTRIC TURBIDITY MEASUREMENTS

	ADDED TO THE BASIC INORGANIC MEDIUM: <sup>2</sup>							
	TRAUMATIC ACID			VITAMIN B <sub>1</sub>		ADE- NINE B <sub>1</sub> + ADEN.		NO ADDI- TIONS
	5000 γ/l	1000 γ/l	100 γ/l	1000 γ/l	100 γ/l	1000 γ/l	100 γ/l EACH	
<i>Scenedesmus obliquus</i>	62.0	41.4	26.2	..	..	..	..	19.4
(6 days after inoculation; pH 6.6)	68.9	45.1	26.9	..	..	..	..	22.2
<i>Sc. bijugatus</i>	99.2	84.8	56.2	..	..	..	..	49.2
(12 days after inoculation; pH 6.6)	93.0	80.6	52.2	..	..	..	..	47.6
<i>Sc. bijugatus</i>	...	94.4	40.6	24.0	37.3	31.1	43.2	22.6
(20 days after inoculation)								
<i>Sc. brasiliensis</i>	141.4	100.0	71.4	..	..	..	..	74.2
(12 days after inoculation; pH 6.6)	118.0	92.2	70.2	..	..	..	..	62.4
<i>Palmellococcus miniatus</i>	115.6	107.0	49.8	..	..	..	..	56.4
(12 days after inoculation; pH 6.6)	102.0	92.6	48.0	..	..	..	..	48.2
<i>Haematococcus pluvialis</i>	...	Fourth	No	Best	Second	No	Third	No
(ring on wall of tube esti- mated after 20 days)			growth			growth		growth

In a purely inorganic medium and under the conditions described here the algae probably produce<sup>14</sup> a limited amount of the growth factors, but not sufficient to sustain a rapid growth.

<sup>1</sup> Work assisted by the Works Progress Administration (Official Project 65-1-07-98, Work Project N-11534).

<sup>2</sup> *Ann. Bot.*, 43, 805-816 (1929).

<sup>3</sup> E.g., *Am. Jour. Bot.*, 27, 161 (1940).

<sup>4</sup> Bold, Harold C., *Jour. Tenn. Acad. Sci.*, 12, 205-212 (1936).

<sup>5</sup> *Proc. Nat. Acad. Sci.*, 23, 174-176 (1937).

<sup>6</sup> *Am. Jour. Bot.*, 25, 498-501 (1938).

<sup>7</sup> *Science*, 90, 329 (1939); the traumatic acid used in these experiments was obtained through courtesy of these authors.

<sup>8</sup> The basic inorganic medium consisted of  $\frac{1}{5}$  Hoagland solution plus minor elements, with the exception of copper (*Univ. Cal., Coll. Agr. Cir.* 347, 36-37, December (1938)). Glass-distilled water was used throughout. Agar was not used in the experiments described here because it was found to be an unsuitable medium for the demonstration of the action of organic growth factors. Inoculations were made by using 1 cc. of a cell

suspension. The cultures were in culture tubes (15 cc. of medium) in indirect daylight in an air-conditioned greenhouse (constant temperature, 26°C.).

<sup>9</sup> These algae were obtained through courtesy of Dr. C. B. van Niel, Pacific Grove, Calif.

<sup>10</sup> Schopfer, W. H., *Compt. Rend. Soc. Phys. Hist. Nat. Geneve*, **51**, 26 (1934).

<sup>11</sup> Kögl, F., and Haagen-Smit, A. J., *Zeit. Physiol. Chem.*, **243**, 209-226 (1936); Bonner, J., *Science*, **85**, 183-184 (1937); Robbins, W., and Bartley, M., *Ibid.*, **85**, 246 (1937).

<sup>12</sup> Obtained through courtesy of Dr. Florence Meier Chase, Smithsonian Institution.

<sup>13</sup> Bonner, D. M., and Haagen-Smit, A. J., *Proc. Nat. Acad. Sci.*, **25**, 184-188 (1939).

<sup>14</sup> The presence of vitamin B<sub>1</sub> in marine algae was reported by E. R. Norris, M. K. Simeon and H. B. Williams, *Jour. Nutrition*, **13**, 425-433 (1937).

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## SOME TRABECULATE CODIUMS (INCLUDING TWO NEW SPECIES)

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Trabeculae ("Balken," "Cordons cellulosiques," etc.), as applying to protrusions from the "wall" into the cavity of a septate or unseptate coenocyte, occur particularly in species of *Caulerpa* and *Dictyosphaeria*, both genera of the general group of the Siphonales, to which the genus *Codium* also belongs. Thus far they have not been described for any of the numerous species of *Codium*. In a few species of *Codium*, however, various types of projections have been noticed extending from the inner portions of the apical membrane of the utricles into the general cavity of the coenocyte. O. C. Schmidt ("Biblioth. Bot.," Heft 91, p. 27, figure 7 (1923)) shows tips of utricles of his extended and probably composite *Codium adhaerens* which have the inner portions of the membrane alveolate, with inwardly extending anastomosing plates (as shown in section in the lowermost tip under his figure 7). Such sculpturing of the inner layers of the apical membrane has been seen by me in a number of species of both *Tylecodiums* and *Schizocodiums*, often sufficiently constant to seem characteristic of the particular species. M. A. Howe (in Britton and Millspaugh, "The Bahama Flora," p. 617 (1920)) has described a variety, or form, *cribosum*, of *Codium intertextum* Collins and Hervey, but this alveolation seems to be really a character of the mature utricles of this species (which is included under the extensive aggregate of *Codium adhaerens* by O. C. Schmidt). These alveolar inner surfaces of the apical membrane have been little noticed and often are not to be seen except on careful illumination. From the surface, under proper lighting of fresh utricles or those swollen to nor-

mal size and shape, they look like the sieve plates of the higher plants. It seems best to regard them as lamellate anastomosing projections, rather than "pitting," and to consider them under the head of complex trabeculae.

Another type of trabeculate growth is shown in the peculiar local thickening of the inner apical membrane of the *Codium Schmidtii* Vouck (V. Vouck, *Acta Bot., Inst. Bot. Univ. Zagrabensis*, 10, 9-12, plate 1, text figures 1-4 (1935)), who states, accurately so far as I know, that "such a peculiarity of utricle structure has never been recorded." The apical membrane of the utricle thickens, but at the center the thickening projects into the cavity of the utricle in the form of a boss, or button. Unfortunately for this proposed new species of Vouck, I find that the utricles of the type specimen of *Codium Muelleri* of Kuetzing show the same thickened structure, although neither Kuetzing (*Tab. Phyc.*, 6, 34, plate 95, II (1856)) nor O. C. Schmidt (loc. cit., 51, 52) figures it. Kuetzing has nothing in his description to suggest that, "keen-eyed" as he undoubtedly was, he had noticed anything of this inwardly projecting blunt umbo, but Schmidt (loc. cit., p. 51) evidently saw it and failed to interpret it properly, although he quotes Areschoug's statement (*Act. Reg. Soc. Scient.*, ser. 3 (1), 368, Upsala (1854) sub *Codium tomentosum australasiacum* Aresch.) concerning an apical "mamilla" on the utricle separated by a transverse septum from the utricle itself. O. C. Schmidt seems to regard this "mamilla" to be due to an illusive appearance in the collapsed broad utricles of the species, not properly swollen out. Vouck's interpretation of the structure agrees with my own, but his binomial *C. Schmidtii* must, therefore, be relegated to the synonymy of *C. Muelleri*. It may be of interest to notice that *C. Schmidtii* Vouck is a topotype of *C. Muelleri* Kuetz., since both were collected on shores of the Lefever (or Lefebre) Peninsula by Ferdinand Mueller, the former in "July, 1882," the latter in "July, 1852."

When one reads the descriptions of the utricles of *Codium globosum* of A. H. S. Lucas (*Proc. Linn. Soc. of New South Wales*, 52, (pt. 4), 558, plate 12, figure 4 (1927)) one will not find any mention of the scattered, short, broadly conical trabeculae projecting from the inner surface of the apical

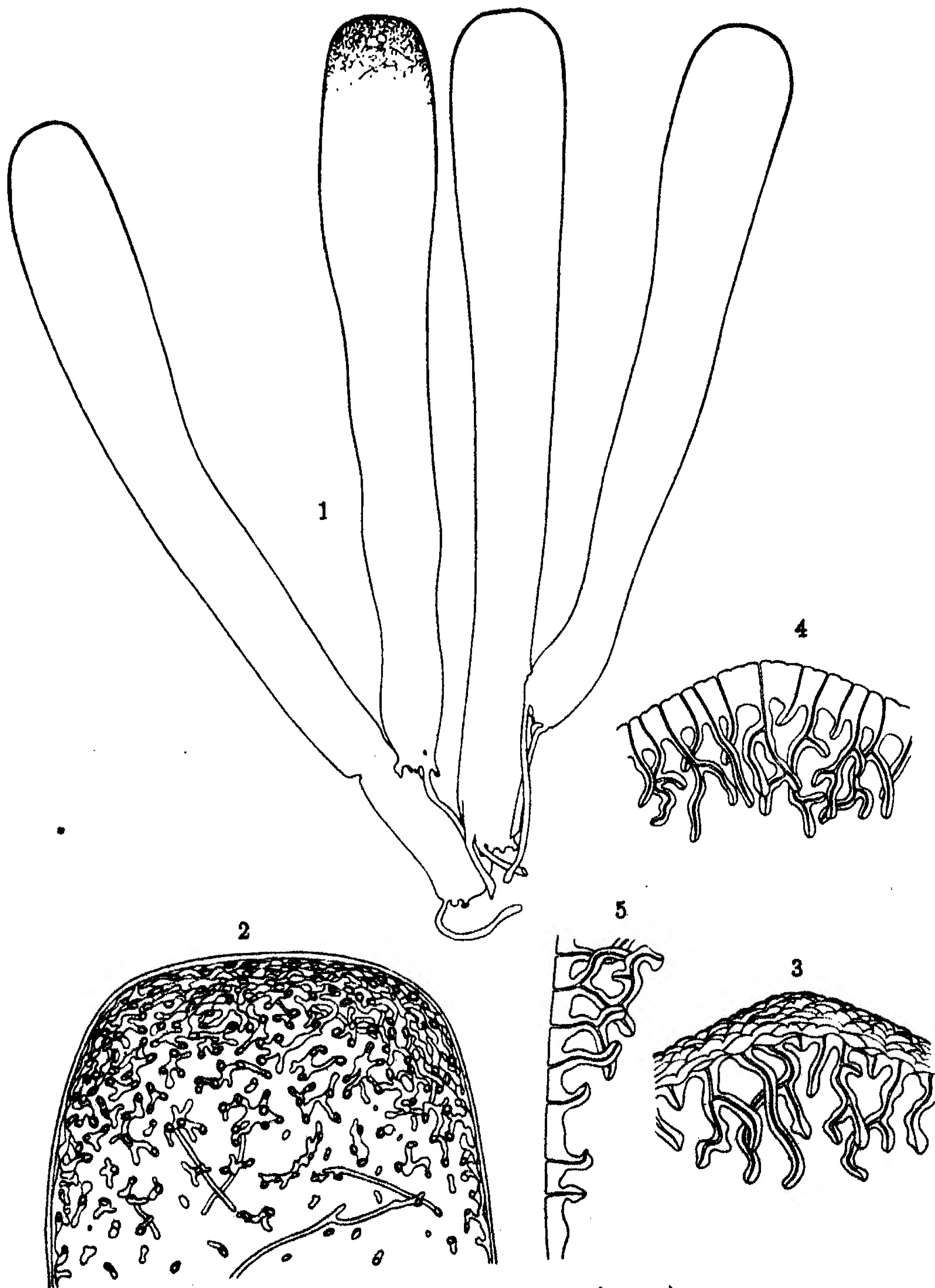
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#### EXPLANATION OF PLATE

##### *Codium Cranwelliae* Setchell

1. Group of utricles to show size, shape and branching. Trabeculae are represented in only one while the rest are left blank.  $\times 25$  diam.
2. Uppermost portion of utricle with trabeculae from the surface and, in part, in optical section.  $\times 125$  diam.
3. Thick section of extreme tip of utricle, showing outer surface and trabeculae.  $\times 250$  diam.
4. Thin section of extreme tip of utricle.  $\times 250$  diam.
5. Thin section of lower part of trabeculate zone of side of upper part of utricle.  $\times 250$  diam.





(See explanation of plate on opposite page)

membrane. A careful examination of the utricles of specimens of the type collection, however, does show them.

While the "trabeculae" of the three species thus far listed may seem too insignificant to warrant applying this term to them, the structure seems more definitely worthy of the name as applied in the case of the two species now to be described, viz., *Codium phasmaticum* from Hawaii and *C. Cranwelliae* from New Zealand.

*Codium phasmaticum* sp. nov.

*C. thallo applanato, difformi, inferne arcte adhaerente, margine lente undulato, spongioso, 3-5 cm. diam., circum 0.5 cm. crasso, laetevirente; utriculis laxe corymboso-ramosis, dimorphis, (a), macro-stenophysis, cylindricis, 62.5-93.75  $\mu$  latis et 500  $\mu$  usque ad 1 mm. altis, (b), macro-euryphysis, obovato-pyriformibus, superne globoso-intumescens, 125-250  $\mu$  latis, 500  $\mu$  usque ad 625  $\mu$  altis; membrana apicali usque ad 5  $\mu$  crassa, intus 1-2 usque ad 7-8 trabeculis cylindricis, simplicibus, gracilibusque usque ad 15  $\mu$  longis et 2.5-3  $\mu$  crassis adornatis; sub apice 62.5-65  $\mu$ , pilis in singulo usque ad 3-4 verticillis, circumdatis; gametangiis adhuc non visis.*

*In saxis littoralibus, apud "Laupahoehoe," in insula "Hawaii," a W. A. Setchell lecto, No. 10034 (spec. typicum Hb. Univ. Calif. 468720).*

Only a single specimen was found on rocks which were almost bare of any Algae. Each utricle, however, shows the relatively long and slender, although sparse, inwardly projecting processes whose structure is that of the simpler trabeculae of *Caulerpa*, or even more like those of certain species of *Dictyosphaeria* (cf. Weber van Bosse, "Nuova Notarisia," ser. 16, 144, Oct. (1905), Siboga Exp., Mon. LIX a, 64 (1913); Boergesen, "Mar. Alg. Danish W. I.," 1, 39 (1913); Setchell, "Univ. Calif. Pub. Bot.," 12, 79 (1926)).

*Codium Cranwelliae* sp. nov.

"A new *Codium*," Cranwell and Moore, *Trans. Roy. Soc. N. Z.*, 67, 399 (1938).

*C. thallo compresso-ovoideo-globoso, 0.5-5 cm. longo, 0.5-3 cm. lato, 0.5-2.5 cm. alto, erecto, superne libero, per basim moderate latam affixo, nitente laetevirente et spongioso; filamentis internis 35-40  $\mu$  latis, dense intertextis; utriculis superficiem liberam totam investientibus, parce ramosis, elongato clavatis, megistophysis, 5000  $\mu$  usque ad 6500  $\mu$  longis, 625  $\mu$  latis, membrana apicali, et subapicali leviter incrassatis et trabeculas numerosas, simplices ramosasve emittente; pilis deuntibus; gametangiis nondum visis. (Cf. plate, p. 445.)*

*In rupis concavis, zonis medio-littoralibus, in insulis "Poor Knights," N. Z., No. 205, 206, 207, Cranwell et Moore, et ab undis rejectis, "Long Beach, Bay of Islands," N. Z., No. 182, 791, 1557, V. W. Lindauer. (Spec. typicum in Hb. Univ. Calif., No. 564498).*

*Codium Cranwelliae* is a truly remarkable species. It has the habit and the huge ("megistophyse") utricle associated with the *Bursa* group within the genus. Its utricles are distinctly branched although slightly, and this is true even of *Codium Bursa* itself although O. C. Schmidt ("Bibliotheca Botanica," Heft 91, pp. 23, 24, 25 (1923)) in his monographic study of *Codium* distinguishes the "*Adhaerentia*" as having the "Blasen reich verzweigt" and the "*Bursae*" as well as the other two sections as having "Blasen stets unverzweigt." I find, however, that *Codium Bursa* (L.) Ag. and *C. mammillosum* Harv. have utricles distinctly although only slightly branched and many species included in Schmidt's "*Adhaerentia*" other than *C. adhaerens* in Schmidt's extended sense, can scarcely be said to have the utricles really "richly" branched. In habit, *Codium Cranwelliae* is truly of the *Bursa* group, being cushion-shaped and with a limited basal area of attachment, even somewhat more so than *C. spongiosum* Harv. of Australian waters, which it closely resembles, except that it seems more densely packed with filaments in its interior, has a narrower attachment, and has the conspicuously trabeculate utricles. From *Codium Perrinae* A. H. S. Lucas (*Proc. Linn. Soc. N. S. W.*, 60, 203, figure 4 (1935)), apparently closely related to *C. spongiosum*, it differs in lack of crescentic habit and the longer and trabeculate utricles not thickened at the apex.

The utricles in *Codium Cranwelliae* are very long (up to 6 or 7 mm.) and are "megistophyse" in one sense but they are not as broad as those of the truly megistophyse species of the *C. mammillosum* group of Australia, Japan and South Africa, which approach a maximum of 2-3 mm. in diameter. It is, however, the occurrence of undoubted trabeculae, not only crowded but with one or more branches, which sets off *C. Cranwelliae* from all other *Codiums* thus far described. This has led me to review the various *Codiums* which have been accumulating for some years in my studies of this genus and which had led me to survey all the species known to me to have "projections" inwardly from the apical or subapical membranes and consider them under the head of "trabeculate" *Codiums* and to associate these structures with the various inwardly projecting cylindrical protrusions of the inner cell walls in the various members of the Siphonales. The structure of the trabeculae in *Codium* seems to be identical with those in *Caulerpa*, in that a distinct core (or tube?) is present. The trabeculae are always on the apex of the cavity of the utricle, protruding from the inner surface of the apical or subapical membranes, limited in growth, simple or branched, and ending free. Oltmanns ("Morph. u. Biol. d. Algen," I (Ed. 2), 413-415 (1922)) summarized the literature and opinions as to structure and function of the trabeculae in *Caulerpa*, up to his time of writing. As to what their function may be in the utricles (and only in the utricles) of *Codium*, the writer has as yet no suggestions, especially since he has seen only dead plants. The fact of their occurrence and particularly their luxuri-

ant development in *Codium Cranwelliae* may add information to be considered in any attempt to solve the problem.

## STUDIES IN MINERAL METABOLISM WITH THE AID OF INDUCED RADIOACTIVE ISOTOPES. IV—MANGANESE\*

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This communication is a report of a test study of the suitability of radioactive manganese for biological tracer investigations. The requirement for manganese of the animal organism is very small. Consequently, a high specific radioactivity is required in the samples in order to keep the test dosages within physiological limits.

Various investigators have demonstrated that manganese is essential for the health and well-being of the animal organism.<sup>1, 2, 3, 4</sup> However, little is known about its specific biological functions. Orent and McCollum<sup>5</sup> found that manganese aids lactation and prevents degeneration and atrophy of the testes in the rat. In the chick, it is markedly effective in preventing the development of the bone condition known as perosis.<sup>6</sup> Manganese has been found to serve as an activator of certain enzymes, notably arginase,<sup>7, 8</sup> phosphoglucomutase,<sup>9</sup> and certain peptidases.<sup>10</sup>

The results reported here demonstrate that radioactive manganese may be usefully employed in the elucidation of many problems connected with its metabolism.

*Experimental Methods.*—The isotope  $Mn^{54}$ , with a half life of 310 days,<sup>11</sup> was employed in this investigation. It was prepared in the Radiation Laboratory of the University of California by bombardment of iron with deuterons. The reaction involved is:



Radioactive manganese was isolated from the residue after extraction of the radioactive iron. Traces of radioactive phosphorus formed from iron phosphide were removed by dissolving the iron-free residues in 0.5M  $HNO_3$ , adding small quantities of  $NaH_2PO_4$  to serve as a carrier, and precipitating twice with a solution of  $Bi(NO_3)_3$  in 0.5M  $HNO_3$ . The filtrate was evaporated to dryness, dissolved in 16M  $HNO_3$ , and the manganese precipitated as  $MnO_2$  with a few crystals of  $KClO_3$ . The  $MnO_2$  was filtered off on a Jena sintered glass filter, then dissolved with  $H_2O_2$ , and reprecipitated twice by

the above procedure to free it from traces of radioactive cobalt that were also present in the original residues.

Finally the  $\text{MnO}_2$  was dissolved in dilute  $\text{HCl}$ , evaporated to dryness to remove excess acid and then dissolved in enough water to give a concentration of about 1 mg. of manganese per ml. of solution. The radioactivity of the resulting preparation was of the order of 0.1  $\mu\text{c.}$  per mg. of Mn. All radioactivity measurements were made with a thin copper wall Geiger-Müller counter and a scale of eight circuit.

TABLE 1  
EXCRETION OF LABELED MANGANESE

TIME, HOURS	ORAL ADMINISTRATION, PER CENT OF TOTAL DOSE	INTRAPERITONEAL INJECTION, PER CENT OF TOTAL DOSE
	Feces	Feces
0-23	4.2 $\pm$ 0.26	*
23-48	50.2 $\pm$ 0.45	53.9 $\pm$ 0.60
48-75.5	39.9 $\pm$ 0.40	36.8 $\pm$ 0.35
Total excreta	97.2† $\pm$ 0.68	90.7 $\pm$ 0.70

\* Amount found was not statistically significant.

† Total excreta includes 2.9 per cent labeled manganese found in the urine in the time interval between 11 and 23 hours. This was the only statistically significant amount found in any of the urine samples.

For the metabolic tests, 1-ml. doses of the labeled manganese chloride (1 mg. Mn) were administered to each of two rats weighing about 160 gm., one by stomach tube, the other by intraperitoneal injection. During the experiment, the animals were maintained on a synthetic control diet.

The feces and urine were obtained separately at the desired intervals through use of glass separators.<sup>12</sup> At the end of 75.5 hours, the animals were anesthetized with ether, blood was drawn by cardiac puncture and the animals were sacrificed. The various organs were dissected out, the skin was removed and the muscles and bone were separated by boiling the residual carcass in 1:5  $\text{NH}_4\text{OH}$ , and then allowing the solution to stand for several days.

All tissues were dried and dry ashed at 500°C. One mg. of inactive manganese as the sulfate was added to each ashed sample as a carrier for the radioactive Mn. The ash was dissolved in a minimum of dilute  $\text{HCl}$  and filtered. The total filtrate was evaporated to dryness with concentrated  $\text{HNO}_3$  three times to remove the chloride. The residue was then dissolved in hot concentrated  $\text{HNO}_3$ , and a few crystals of  $\text{KClO}_3$  were added to precipitate the  $\text{MnO}_2$ . The precipitate was filtered off, another mg. of inactive Mn was added to the filtrate and  $\text{MnO}_2$  was again precipitated. This second precipitate was collected on the same filter paper so that all of the radioactivity in a single tissue was on the one filter paper.



In the case of the bones, it was necessary first to separate the manganese from the large amounts of calcium present. The bone ash was dissolved in dilute HCl and evaporated to dryness. The chlorides were dissolved in water in an Erlenmeyer flask and  $\text{NH}_4\text{Cl}$  was added to the neutral solution. A freshly prepared  $(\text{NH}_4)_2\text{S}$  solution was added to precipitate the manganese sulfide. The flask was filled with boiled water and stoppered. After standing for 12 hours, the precipitate was filtered off. The  $\text{MnS}$  was dissolved in a small amount of dilute HCl, and then the  $\text{MnO}_2$  was separated by the same procedure as in the case of the other tissues. An aliquot of the original radioactive  $\text{MnCl}_2$  solution was treated in the same manner as the test samples to serve as a standard for comparison of radioactivity measurements.

The total radioactive manganese recovered from each animal was computed from the measured activities of the excreta and of all the tissues. The recovery was 96 per cent in the case of oral administration, and 78 per cent in the case of intraperitoneal injection, so that the data have been corrected by the factor  $100/96$  and  $100/78$ , respectively, to make them comparable.

*Results.*—The course of excretion of the labeled manganese over the 75.5 hours is shown in table 1. Most of the manganese is excreted in the feces whether it is administered orally or by injection. This agrees with Skinner, Peterson and Steenbock,<sup>13</sup> who have reported that from 80 to 99 per cent of orally fed manganese was excreted in the feces, depending upon the amount ingested. Except for statistically insignificant traces, labeled manganese was found in the urine only in the second collection period of the animal given the dose orally.

From the data it is not possible to decide to what extent manganese is absorbed from the gastro-intestinal tract when it is administered orally, although it is probable that it is small. When administered intraperitoneally, 90.7 per cent of the labeled manganese appeared in the feces, showing that there is a preferential excretion into the alimentary tract.

Table 2 shows the distribution of the labeled manganese that was retained by the animal. When administration was oral, the 2.8 per cent retained manganese was found in the liver, bone, muscle and blood, the liver showing the largest uptake.

When administration was by injection, the retained manganese was found in the skin, bone, liver, muscle, small intestine and stomach, the skin and bone showing rather large amounts. Other tissues showed no significant amount. In general, these observations also agree with the findings of Skinner, Peterson and Steenbock.<sup>13</sup>

The manganese found in the stomach and small intestine possibly represents manganese in the process of being excreted. Muscle and skin, apparently, are important sites for the storage of manganese that is absorbed,



especially as these tissues represent a large portion of the mass of the animal. Bone and liver also seem to be important in the storage of manganese. The manganese found in the liver may be indicative of its excretion into the bile, or it may be connected in some manner with the activation of certain enzymes found in the liver.

*Summary.*—1. Radioactive manganese,  $Mn^{54}$ , is suitable for "tracer" studies on the metabolism of manganese.

2. On a normal control diet the rat excreted over 90 per cent of the manganese within 75 hours, when administered either by stomach tube or by intraperitoneal injection.

3. Very little, if any, of the absorbed manganese is excreted in the urine.

4. Liver, bone and muscle take up appreciable quantities of the ab-

TABLE 2  
DISTRIBUTION OF LABELED MANGANESE  
(In Per Cent of Total Dose, 75.5 Hours after Administration)

TISSUES	ORAL ADMINISTRATION			INTRAPERITONEAL INJECTION		
	WEIGHT, GM.	CONTENTS IN WHOLE TISSUE	CONTENTS PER GM., FRESH WEIGHT	WEIGHT, GM.	CONTENTS IN WHOLE TISSUE	CONTENTS PER GM., FRESH WEIGHT
Muscle	95	$0.7 \pm 0.12$	$0.007 \pm 0.0012$	97	$0.8 \pm 0.16$	$0.008 \pm 0.0016$
Bone	8.6	$0.7 \pm 0.11$	$0.081 \pm 0.013$	9.8	$2.0 \pm 0.17$	$0.20 \pm 0.017$
Skin	30.66	*	*	28.94	$3.7 \pm 0.22$	$0.13 \pm 0.008$
Whole blood	5.14	$0.5 \pm 0.14$	$0.097 \pm 0.027$	5.07	*	*
Heart	0.50	*	*	0.56	*	*
Liver	6.49	$0.9 \pm 0.15$	$0.14 \pm 0.023$	8.54	$1.2 \pm 0.20$	$0.14 \pm 0.023$
Small intestine	5.37	*	*	6.36	$0.8 \pm 0.27$	$0.13 \pm 0.042$
Large intestine	1.13	*	*	1.23	*	*
Stomach	4.75	*	*	3.67	$0.8 \pm 0.27$	$0.22 \pm 0.074$
Spleen	0.35	*	*	0.89	*	*
Kidney	1.38	*	*	1.36	*	*
Lung	0.88	*	*	1.16	*	*

\* Radioactivity measurements made, but amounts found were not statistically significant.

sorbed manganese. Other tissues may take up varying amounts of the manganese, due to storage or to processes of excretion.

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## A REVERSION TO WILD-TYPE ASSOCIATED WITH CROSSING-OVER IN *DROSOPHILA MELANOGASTER*

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In a study of two alleles of lozenge eye in *Drosophila melanogaster*, a low frequency of reversion to the wild-type has been observed. The reversion involves the color and structure of the eye, and also the genital tracts which are abnormal in the mutant females.<sup>1</sup> In each case in which the reversion has occurred, crossing-over between the X-chromosomes has also occurred.

Glossy and spectacle are sex-linked, recessive mutants, alleles of lozenge, which were induced by irradiation. Glossy (*ls<sup>g</sup>*) individuals have eyes which are blood-red in color, with fused facets making a glossy surface. Spectacle (*ls<sup>s</sup>*), as reported by Dr. J. T. Patterson,<sup>2</sup> is characterized by a light brown color of the eye, and the facets are run together to cause a smooth surface of the eye. In the compound, heterozygous glossy-spectacle females, glossy is more dominant in its expression; and the character of the eye is nearly that of homozygous glossy. Spectacle is associated with the *dl-49* inversion, and the mutant gene is located within that inversion. Glossy is also located within an inversion which is very similar to, probably the same as, the *dl-49* inversion.

From the mating of *ls<sup>g</sup> Bx/ls<sup>s</sup> f* females to *ls<sup>g</sup> Bx* males, a total of 5584 offspring have been inspected. Most of the offspring were of the expected types. Males were glossy or spectacle. Females were phenotypically

alike, although genotypically they were either homozygous glossy or heterozygous glossy-spectacle. Eleven of the 5584 offspring, two males and nine females, were wild-type for the mutant eye and genital traits. The wild-type traits persist whether the individuals are heterozygous for spectacle or glossy. Two of the exceptional flies were tested for crossing-over with a combination of genes in a non-inverted *X*-chromosome. The results indicated that the inversion had not been lost.

The appearance of each exceptional, wild-type offspring was associated with crossing-over. Ten of the exceptional offspring were Beadex (*Bx*), and carried, therefore, the right end of the glossy-bearing *X*-chromosome. The other one, forked (*f*), probably was the result of a double crossover. In a test-cross using yellow and Hairy wing as markers for the left end of the chromosome ( $\gamma$  *Hw lz<sup>s</sup> f/lz<sup>s</sup> Bx*), two out of 305 offspring were of the reversed type. One, a male, was  $\gamma$  *Hw Bx*; and the other, a female, proved to be heterozygous for that combination. The flies showing the reversion to wild-type have an *X*-chromosome which is composed of the right end of the glossy-bearing chromosome and the left end of the spectacle-bearing chromosome. Moreover, the associated crossing-over occurs within the inverted regions. The loci for miniature (*m*) and vermilion (*v*) are located in that order within the inverted regions. From the mating of *v lz<sup>s</sup> f/m lz<sup>s</sup> Bx* females to glossy males, two exceptional males occurred among 1285 offspring, and both were *v Bx*. Apparently the crossover occurs between *v* and one of the alleles of lozenge.

Although crossing-over seems to be associated with the reversion, and one crossover type appears, the complementary type has not been recovered. It is not known whether the failure to recover the complementary type is due to the inability of that type to live, or to the inability of the observer to recognize the combination. Glossy is almost completely dominant over spectacle. The presence of glossy and spectacle in one chromosome, with either glossy or spectacle in the other chromosome, may produce a phenotype so similar to homozygous glossy and the compound glossy-spectacle that it will be difficult, if not impossible, to differentiate the three genotypes.

It does not seem possible to explain the reversions on the basis of mutations in the genic sense. The exceptional type has not appeared among the offspring of either homozygous glossy or spectacle; nor has it been observed among the offspring from the females heterozygous for either glossy or spectacle and lozenge. Lozenge is present in a chromosome which has no inversion. The only mating which has given the exceptional, wild-type offspring has been the compound glossy-spectacle. Each of the mutants is present in an inversion, and the inversions are at least almost identical. Under such conditions, crossing-over is expected to occur throughout the chromosome. The crossing-over associated with the reversion occurs

within the inverted region, and as shown by the appearance of the  $v Bx$  crossover type involves the region near the alleles glossy and spectacle.

Although crossing-over is an active factor in the reversion of the alleles to the wild-type, it is not possible as yet to determine the exact nature of the phenomenon. The condition can be a case of unequal crossing-over;<sup>3</sup> but it can as likely be a case which involves the "repeat" hypothesis developed by Bridges,<sup>4</sup> in which different primary loci of the chromosome are involved in the expression of the two mutants.

*Summary.*—1. A reversion to wild-type occurs with a frequency of 0.2% among offspring from a female which had glossy on one and spectacle on the other X-chromosome. Both traits are recessive and are allelic to lozenge.

2. The reversion is always associated with crossing-over.

3. The associated crossing-over occurs within the inverted regions of the chromosome, and at or near the lozenge locus.

4. The suggestion is made that the results probably involve either unequal crossing-over or crossing-over between "repeats."

<sup>1</sup> Oliver and Green, *Anat. Rec.*, 75 (Supp.), 100-101 (1939).

<sup>2</sup> Patterson and Muller, *Genetics*, 15, 495-578 (1930).

<sup>3</sup> Sturtevant, *Ibid.*, 10, 117-147 (1925).

<sup>4</sup> Bridges, *Jour. Heredity*, 29, 11-18 (1938).

## HD 167362, AN OBJECT SIMILAR TO CAMPBELL'S HYDROGEN ENVELOPE STAR

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Campbell's famous hydrogen envelope star<sup>1</sup> consists of a Wolf-Rayet nucleus of type WC8, which is surrounded by a nebula showing strong forbidden [N II] lines; in fact, the two red [N II] lines contribute more than  $H\alpha$  to the red color of the envelope. This association seems rather peculiar, the exciting Wolf-Rayet nucleus showing no trace of nitrogen,<sup>2</sup> whereas the nebulosity around it is very rich in nitrogen.

We have found that spectroscopically HD 167362 (CD  $-30^{\circ}$  15469; MWC 288;  $m = 11.8$ ) is a similar object. Its distance is too great to resolve the nebula, and the widths of the lines belonging to the nucleus are not as much broadened as they are in Campbell's star. The bright lines were discovered by Keeler<sup>3</sup> at the Harvard Observatory and were first de-

scribed by Miss Fleming,<sup>3</sup> who called attention to the similarity with  $\eta$  Carinae. Miss Cannon<sup>4</sup> listed the spectrum among the P Cygni stars, and recorded the presence<sup>5</sup> of [O III] 5007 and 4959, and of other faint emission lines.<sup>6</sup> Miss Hoffleit<sup>7</sup> found no change in the apparent magnitude of the star. Merrill and co-workers<sup>8</sup> found that  $H_\alpha$  is bright. C. Payne Gaposchkin and S. Gaposchkin<sup>9</sup> list the star among objects possessing forbidden [Fe II], but the source of their observation is not given. Our plates do not show [Fe II]. Apparently, no detailed study of this interesting spectrum has thus far been published.

Five spectrograms were obtained at the McDonald Observatory, from April 24 to May 2, 1940; three were obtained with the quartz prisms (dispersion 100 Å/mm. at  $\lambda$  3933) and two with the glass prisms (dispersion 50 Å/mm. at  $\lambda$  3933).

The spectrum consists of a weak continuum, with maximum intensity near  $\lambda$  4300, on which are superimposed strong, bright lines. The forbidden lines and the lines of H seem to be a little sharper than the He I lines and He II 4686. Still broader features belong to C III and C IV. The measured lines are collected in table 1.

The object consists of a Wolf-Rayet nucleus surrounded by a nebula, and we distinguish three groups of lines, which we shall designate as *A*, *B* and *C*.

*A. Lines Originating in the Nucleus Only.*—This is the case of the broad lines of He II, C III, C IV and O III; the Wolf-Rayet nucleus is a typical carbon star; according to the intensity ratios adopted for classification purposes, the spectral type is probably later than the latest type WC8 in Beals' sequence. Since the band width is much smaller than 10 Å and since He I plays certainly an important rôle in the nuclear part of the spectrum (see group *C*), we should be inclined<sup>10</sup> to assign the central star to type WC9. There is no trace of N II, N III, N IV or N V. Several lines of the nucleus have P Cygni character.

*B. Lines Originating in the Nebula Only.*—The forbidden lines of the following elements (in order of decreasing intensity) are observed in the nebula: [N II], [O II], [O III], [S III], [O I]. The transitions of the nebular type are much stronger than those of the auroral type (for example, in [O III] and [N II]). This is the usual thing in planetary nebulae. The transeauroral transitions of [S II] may be present, but are blended with C III 4069 and O II 4076. The mean ionization from strong lines of [N II] and [O II] is around 14 volts; but the forbidden lines of neutral [O I] and doubly ionized [O III] and [S III] are fairly strong.

*C. Lines Belonging to the Nucleus and to the Nebula.*—The lines of He I and H are not exclusively of nebular origin. This conclusion is based upon the following facts: (1) The lines of [N II] and [O III] are sharper than those of He I, as may be seen from a comparison of He I 5876 and N II

TABLE 1

## SPECTRUM OF HD 167362

STAR			IDENTIFICATION		
$\lambda$	Int.	Element	$\lambda$	Int.	Notes
3609.5	1 $n$	C III	3609.61	5	
		C III	3608.96	4	
3682.7	1	H <sub>20</sub>	3682.81		
3687.0	1	H <sub>19</sub>	3686.83		
3691.3	1	H <sub>18</sub>	3691.56		
3697.0	1	H <sub>17</sub>	3697.15		(1)
3703.8	1-2	H <sub>16</sub>	3703.85		(1)
3711.9	2	H <sub>15</sub>	3711.97		(1)
3715.	0 $n$	O III	3715.08	6	
3721.8	2	H <sub>14</sub>	3721.94		(1)
3725.9	8	[O II]	3726.2		
3728.6	6	[O II]	3729.1		
3734.2	2	H <sub>13</sub>	3734.37		
3750.4	2-3	H <sub>12</sub>	3750.15		
3755.2	1	O III	3754.67	7	
3761.	1 $n$	O III	3759.87	9	
3770.9	3	H <sub>11</sub>	3770.63		
3791.9	1 $n$	O III	3791.26	6	
3797.9	3-4	H <sub>10</sub>	3797.90		
3819.4	2	He I	3819.61	4	
3835.4	4	H <sub>9</sub>	3835.39		
3867.6	0	He I	3867.46	2	
3888.7	4	He I	3888.65	10	(4)
3889.1	4	H <sub>8</sub>	3889.05		
3970.2	6	H <sub>7</sub>	3970.08		(1)
4026.3	2	He I	4026.19	5	
4057.0	1 $n$	C III	4056.06	5	
4068.7	3 $n$	C III	4067.87	9	(1)
		C III	4068.97	9-10	
		C III	4070.30	10	
		[S II]	4068.5		
4075.7	1-2	[S II]	4076.5		
		O II	4075.87	10	
4101.8	8	H <sub>6</sub>	4101.75		
4120.	1	He I	4120.81	3	
4144.	0	He I	4143.77	2	
4155.4	1	C III	4156.50	4	
		C III	4152.43	3	
4183.4	2A	C III	4187.05	10	(1)
4186.0	2E				
4340.7	10	H <sub>7</sub>	4340.48		(1)
4362.7	1	[O III]	4363.21		
4388.	1	He I	4387.93	3	
4468.7	3A	He I	4471.48	6	(1)
4471.3	3E				
4516.7	1	C III	4516.5	4	



4651.	4nn	C III	4647.44	20	(1, 2)
		C III	4650.26	19	
		C III	4651.48	18	
4658.8	2n	C IV	4658.64	5	
4667.	1n	C III	4665.90	6	
4686.	1n	He II	4685.81		
4861.3	10	H $\beta$	4861.34		
4958.9	4s	[O III]	4958.91		
5006.9	7s	[O III]	5006.84		
5696.	3n	C III	5696.0	8	
5755.3	3s	[N II]	5755.0		
5802.	0n	C IV	5801.51		(3)
5876.0	5	He I	5875.62	10	
6300.	2	[O I]	6300.2		
6311.	3	[S III]	6310.2		
6548.	7	[N II]	6548.4		
6563.	15	H $\alpha$	6562.82		
6584.	10	[N II]	6583.9		
6678.	1	He I	6678.15	(6)	

Identifications in square brackets designate forbidden transitions.

1—Line showing the P Cygni character.

2—Broad line extending over 4.1  $\text{\AA}$ .

3—For this identification see the discussion of the spectrum of NGC 6543 and its nucleus; P. Swings, *Ap. J.* (in press).

4—Blended.

5755. (2) Several lines of H and He I show P Cygni character, the velocity of ejection being practically the same as that given by the nuclear lines.

The Balmer lines are followed to H $_{20}$  and a strong Balmer continuum extends to  $\lambda$  3450.

The lines of the nucleus and of the nebula seem to have the same radial velocity. The mean value from 18 lines is  $-24$  km./sec. The ejection velocities obtained from He I 4471 and C III 4187 are 176 and 186 km./sec., respectively. Since these values are practically equal we adopt for the ejection velocity 180 km./sec.

The great similarity of HD 167362 to Campbell's star (BD + 30° 3639 = HD 184738) is evident at once. In both cases, the nucleus is a late WC star and the nebula shows very strong [N II] and [O II] lines. In BD + 30° 3639, H $\alpha$  is weaker than the total intensity of the two red [N II] lines, but this may not be true for HD 167362. But it should be remembered that in HD 167362, H $\alpha$  belongs simultaneously to the nebula and to the nucleus; hence the relative contributions of H $\alpha$  and [N II] in the nebula are probably not very different. In both stars the nucleus shows He I lines; the spectroscopic separation between nucleus and nebula is easier in the case of Campbell's star, where the two sources are clearly separated in a large telescope and where the widths of the lines are very different. In Campbell's star, He I is practically absent from the nebular part of the spectrum, while

it is present in the nucleus. [O III] is strong in HD 167362, and very weak in Campbell's star.

The striking association in HD 167362 and BD + 30° 3639 of a carbon nucleus with a nitrogen envelope suggests that a comparison with NGC 6543 would be interesting, despite the higher excitation prevailing in the nuclear and nebular parts of NGC 6543.<sup>11</sup> This object also shows strong nebular lines of [N II], but its nucleus exhibits both N IV and C IV with similar intensities.

<sup>1</sup> *Astronomy and Astrophysics*, 13, 461 (1894).

<sup>2</sup> Several excellent spectrograms of Campbell's star (BD + 30° 3639) have recently been secured at the McDonald Observatory and agree closely with the description of the spectrum by Wright (*Lick Obs. Pub.*, 13, 220 (1918)); there is no trace of N II, N III, N IV or N V in the nucleus, which is a typical carbon star.

<sup>3</sup> *Ap. Jour.*, 2, 354 (1895).

<sup>4</sup> *Harvard Ann.*, 76, 31 (1916).

<sup>5</sup> *Harvard Circ.*, No. 224 (1921).

<sup>6</sup> Henry Draper Catalogue.

<sup>7</sup> *Harvard Bull.*, No. 892, 20 (1933).

<sup>8</sup> *Ap. Jour.*, 61, 389 (1925); 76, 156 (1932).

<sup>9</sup> "Variable Stars," *Harvard Obs. Monograph*, No. 5, 311 (1938).

<sup>10</sup> Beals has chosen the numbering from WC6 to WC8 so as to allow a certain latitude for new discoveries at either end of the sequence. See *Trans. I. A. U.*, 6, 248 (1938).

<sup>11</sup> P. Swings, *Ap. Jour.* (in press).

## THE SPECTRUM OF RW HYDRAE

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RW Hydrae<sup>1</sup> is an abnormal long-period variable having an unusually small range, of about one magnitude; the maximum photographic magnitude is 9.7 to 9.9 and the minimum 10.8 to 10.9. It has a late-type spectrum upon which are superimposed several bright lines. Miss Cannon's estimates of the spectral type range from K5 to M2; she noticed bright H<sub>β</sub>, H<sub>γ</sub>, and H<sub>δ</sub>.

The spectrum has been investigated by Merrill,<sup>2</sup> who observed, besides the late-type spectrum, several bright lines of H, He I, He II and [O III] (auroral transition λ 4363 only).

This object offers a striking similarity to AX Persei, CI Cygni, Z Andromedae, R Aquarii, T Coronae and others, consisting of a late-type star and of a companion of high excitation. In previous papers<sup>3</sup> we have given a

new discussion of several binaries of this type, using material secured with the 82-inch reflector of the McDonald Observatory. The present note brings similar information concerning RW Hydrae, based on four spectrograms obtained between April 19 and 25, 1940; two were taken with the quartz prisms (dispersion 100 Å/mm. at  $\lambda$  3933) and two with the glass prisms (dispersion 50 Å/mm. at  $\lambda$  3933).

During the interval covered by our observations, the late-type component was of spectral class *M0* or late *K*; compared with the bright lines, the late-type spectrum was much stronger than in AX Persei or CI Cygni, which we observed between September, 1939, and February, 1940. The red component completely obliterates the region above  $\lambda$  4500, so that only strong bright lines may be detected above that wave-length.

The emission lines are collected in table 1. Besides the features observed by Merrill, our spectrograms reveal many permitted O III-transitions, weak [O II], fairly strong [Ne III], weak  $N_1$  (nebular transition of [O III]), weak Si I and Ca II. All the emission lines are sharp.

The Balmer series is clearly seen in emission to  $H_{22}$ , and a strong Balmer continuum extends to  $\lambda$  3300. Many He I lines are present. The Si I line,  $\lambda$  3905, which also appears in AX Persei and CI Cygni, belongs presumably to the red-variable component; it seems rather probable that the weak Ca II lines are also excited in the atmosphere of the red star.

Besides the strong auroral transition of [O III], we observe a very weak nebular transition,  $N_1$ . Thus, the relative intensities of  $\lambda$  4363 and  $N_1$  are of the type found in planetaries of class Pd, such as IC 4997. Similar relative intensities were observed in AX Persei, CI Cygni, Z Andromedae and R Aquarii, which belong to the same group of binaries.

Fourteen permitted lines of O III are identified between  $\lambda$  3265 and  $\lambda$  3962. This spectrum is not excited by Bowen's fluorescence mechanism to any appreciable extent, because we find the lines of the singlet, triplet and quintet systems with the normal intensities of a recombination spectrum. The ionization potential of  $O^{++}$  is 54.6 volts, which is close to that of  $He^+$ , namely, 54.1 volts. The line He II 4686 is strong. So far as we know, no planetary nebula has been found showing such a complete recombination spectrum of O III. For example, in the extensive investigation by Bowen and Wyse<sup>4</sup> the only recombination line of O III which they observed is  $\lambda$  5592 ( $3s^1P^o - 3p^1P$ ); because of the blending with the red component, we are unable to discuss the region of  $\lambda$  5592, but other recombination lines, such as  $\lambda\lambda$  3774, 3791 and 3962 are not found in the table by Bowen and Wyse.

It is possible that the permitted O III lines do not belong to a nebula, but rather to the exciting nucleus. Both the O III and the N III lines, and perhaps also those of He II and partly those of H and He I may belong to a nucleus of type *WN* possessing abnormally sharp lines; such a *WN* nucleus

TABLE 1  
BRIGHT LINES IN RW HYDRAE

STAR			IDENTIFICATION		
$\lambda$	Int.	Element	$\lambda$	Int.	
3266.	1	O III	3265.45	10	
3312.4	0	O III	3312.30	5	
3341.1	1	O III	3340.74	6	
3383.0	1	O III	3382.69	3	
		O III	3383.85	2	
		O III	3384.95	4	
3429.3	1-2	O III	3428.67	3	
		O III	3430.60	4	
3444.0	3	O III	3444.10	5	
3676.01	1	H <sub>22</sub>	3676.36		
3679.41	2	H <sub>21</sub>	3679.35		
3682.55	2	H <sub>20</sub>	3682.81		
3686.57	2	H <sub>19</sub>	3686.83		
3691.38	2	H <sub>18</sub>	3691.56		
3696.91	2	H <sub>17</sub>	3697.15		
3703.77	2-3n	H <sub>16</sub>	3703.85		
		O III	3702.75	5	
		O III	3703.37	5	
3707.13	0-1	O III	3707.24	6	
3711.80	3	H <sub>15</sub>	3711.97		
3714.60	0	O III	3715.08	6	
3721.75	3	H <sub>14</sub>	3721.94		
3724.90	1	[O II]	3726.1		
3734.36	4	H <sub>13</sub>	3734.37		
3750.14	4	H <sub>12</sub>	3750.15		
3753.98	2-3	O III	3754.67	7	
		N III	3754.62	6	
3759.97	2-3	O III	3759.87	9	
3770.72	4	H <sub>11</sub>	3770.63		
3773.87	1	O III	3774.00	6	
3790.82	1	O III	3791.26	6	
3798.09	5	H <sub>10</sub>	3797.90		
3819.71	2-3	He I	3819.61	4	
3835.54	5	H <sub>9</sub>	3835.39		
3868.74	4	[Ne III]	3868.7		
3889.05	7*	H <sub>8</sub>	3889.05		
		He I	3888.65	10	
3905.26	1	Si I	3905.53	10	
3926.0	1	He I	3926.53	1	
3933.0	2	Ca II	3933.68	200	
3961.58	1-2	O III	3961.59	8	
3965.04	2-3	He I	3964.73	4	
3968.0	1	[Ne III]	3967.5		
		Ca II	3968.49	150	
3970.10	7	H <sub>7</sub>	3970.08		
4009.43	2	He I	4009.27	1	
4026.25	2	He I	4026.19	5	

4097.33	2	N III	4097.31	10
4101.79	10	H <sub>δ</sub>	4101.75	
4121.03	1-2	He I	4120.81	3
4143.88	1-2	He I	4143.77	2
4340.49	12	H <sub>γ</sub>	4340.48	
4363.17	4	[O III]	4363.2	
4387.82	4	He I	4387.93	3
4471.63	3	He I	4471.48	6
4685.72	6	He II	4685.81	
4861.4	15	H <sub>β</sub>	4861.34	
4922.	3	He I	4921.93	4
5007.	1	[O III]	5006.84	
5876.	5	He I	5875.62	10
6563.	20	H <sub>α</sub>	6562.82	

\* The violet wing is weaker than the red wing.

NOTE: Identifications in square brackets designate forbidden transitions.

would be surrounded by a nebulosity giving rise to strong auroral [O III], fairly strong nebular [Ne III], weak nebular [O II] and very weak nebular [O III].

In HD 167362, which contains a late *WC* nucleus exciting a surrounding nebula, we found<sup>5</sup> that the nuclear lines are sharper than is usually observed in Wolf-Rayet stars.

Because of the presence of the strong red component, we cannot settle the question of the excitation of N III; neither is there any information regarding the continuous spectrum of the exciting nucleus. No line of carbon was found.

From 20 bright lines, the radial velocity was found to be +14 km./sec. From three absorption lines (Cr I 4289.72, Fe I 4299.24 and Fe I 4325.76), the radial velocity of the late-type star was found to be +15 km./sec. The two components have practically the same radial velocity.

RW Hydrae belongs to the same group of binaries as AX Persei, CI Cygni, T. Coronae, Z Andromedae, R Aquarii, etc. The excitation of its nebular part is lower than in AX Persei and CI Cygni and is rather similar to R Aquarii; but the intensity ratio of the auroral and nebular transitions is larger in RW Hydrae than in R Aquarii.

<sup>1</sup>  $\alpha(1900) = 13^h 28^m 8^s$ ;  $\delta(1900) = -24^\circ 53'$ ; HD 117970; BD  $-24^\circ 10977$ .

<sup>2</sup> *Ap. J.*, 77, 44 (1933).

<sup>3</sup> *Ap. J.*, 91, 546 (1940); also Swings, Elvey and Struve, *Pub. A. S. P.*, (in press).

<sup>4</sup> *Lick Obs. Bull.*, 19, No. 495 (1939).

<sup>5</sup> *Proc. Nat. Acad. Sci.*, 26, 458 (1940).

## COMPOUND MULTIPLICATIVE DIOPHANTINE SYSTEMS

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1. *Simple Multiplicative Systems.*—The new material in this note begins in § 2, for which the methods recalled in this section are a necessary preliminary.

The power product  $x_{i1}^{a_{i1}} \dots x_{in_i}^{a_{in_i}}$ , in which the  $x$ 's are distinct indeterminates and the  $a$ 's are integers  $> 0$ , will be denoted by  $(x, a, n)_i$ . Hence  $(y, b, m)_j, \dots, (z, c, t)_k$  are defined. A *simple multiplicative system* is a simultaneous system of equations, finite in number, of the form

$$\begin{aligned} a_1(x, a, n)_1 &= a_2(x, a, n)_2 = \dots = a_i(x, a, n)_i, \\ b_1(y, b, m)_1 &= b_2(y, b, m)_2 = \dots = b_j(y, b, m)_j, \\ &\dots \qquad \qquad \qquad \dots \qquad \qquad \dots \\ c_1(z, c, t)_1 &= c_2(z, c, t)_2 = \dots = c_k(z, c, t)_k, \end{aligned} \tag{1}$$

in which  $i \geq 2, j \geq 2, \dots, k \geq 2$ , the coefficients  $a_1, \dots, c_k$  are constant integers  $\neq 0$ , and two or more power products in any one row may or may not have indeterminates in common, while at least two power products in different rows have at least one indeterminate in common. A set of such indeterminates, each of which occurs in at least two rows, will be called a *juncture* of (1).

Two methods are available for finding all rational integer values of the  $x, y, \dots, z$  satisfying the system. In the first,<sup>1</sup> what were called reciprocal arrays furnish a systematic, non-tentative algorithm for exhibiting the complete solution in terms of the minimum number of parameters, independently ranging over all rational integers, necessary and sufficient to give the complete rational integer solution of the system. In the second,<sup>2</sup> the solution of any simple multiplicative system is reduced to finding the primitive solutions of a certain associated system of linear diophantine equations, a problem familiar in the classical theory of algebraic invariants.

An unsolved problem of considerable interest is the *a priori* determination of the minimum number mentioned above in the general case. This number quickly runs into the billions even for systems involving as few as 10 power products in a total of 10 indeterminates none of which occurs to a degree exceeding 10. Thus for even apparently innocuous systems it is impossible to exhibit the complete solution explicitly. For certain types of



systems the minimum number has been determined<sup>2</sup> as a function of the number of indeterminates and their exponents.

2. *Compound Multiplicative Systems.*—A power product in which at least one indeterminate occurs only to the first degree will be called *elementary*, and any indeterminate occurring only to the first degree in an elementary product will be called *distinguished*. Let each of the  $ij \dots k$  power products in (1) be elementary, and let  $x_1, \dots, x_i$  be distinguished indeterminates in the respective power products in the first row of (1). If  $x_1, \dots, x_i$  are distinct, we shall say that  $x_1, \dots, x_i$  is a *distinguished set* for the first row, and similarly for all rows of (1). A distinguished set need not be unique.

The concept of a distinguished set for the entire system (1) enables us to extend the methods recalled in § 1 to certain types of simultaneous additive systems. We shall say that the set  $\Sigma$  of all the indeterminates in the distinguished sets for all the rows of (1) is a *distinguished set for the system* (1) provided the conditions (A), (B) are satisfied:

(A) All the indeterminates in  $\Sigma$  are distinct;

(B)  $\Sigma$  has no indeterminate in common with any juncture of (1).

A simple multiplicative system need have no distinguished set; if it has one, that one need not be unique. Provided (1) has two or more distinguished sets, any one of them may be chosen as the point of departure for obtaining the complete solution next described.

If (1) has a distinguished set, we call

$$\begin{aligned} \sum_{p=1}^i a_p(x, a, n)_p &= 0, \\ \sum_{q=1}^j b_q(y, b, m)_q &= 0, \\ &\dots \\ \sum_{r=1}^k c_r(z, c, t)_r &= 0, \end{aligned} \tag{2}$$

a *compound multiplicative system*.

**THEOREM.** *The problem of finding the complete rational integer solution of a compound multiplicative system is reducible to that of finding the complete rational integer solution of a simple multiplicative system; and the complete solution of the compound system exhibits each of the indeterminates in the system as a polynomial with rational integer coefficients in a certain minimum number of parameters ranging independently over all rational integers.*

Denoting the indeterminates in a distinguished set for (2) by small Greek letters, we may rewrite (2) as either of

$$\begin{aligned} \sum_{p=1}^i a_p \alpha_p A_p &= 0, & \sum_{p=1}^i \alpha_p A'_p &= 0, \\ \sum_{q=1}^j b_q \beta_q B_q &= 0, & \sum_{q=1}^j \beta_q B'_q &= 0, \\ &\dots & &\dots \\ \sum_{r=1}^k c_r \gamma_r C_r &= 0, & \sum_{r=1}^k \gamma_r C'_r &= 0, \end{aligned} \quad (3)$$

in which the  $A, B, \dots, C$  are power products, and  $A'_p \equiv a_p A_p, B'_q \equiv b_q B_q, \dots, C'_r \equiv c_r C_r$ . The  $\alpha_p, A'_p, \beta_q, B'_q, \dots, \gamma_r, C'_r$  are considered temporarily as distinct indeterminates. The complete rational integer solution of each equation in the second form of (3) is then obtained by an application of a recent theorem due to Dickson.<sup>3</sup> In each solution, exactly half the total number of indeterminates are expressed as power products in a certain minimum number of integer parameters, each parameter entering only to the first degree, while the remaining indeterminates are expressed as polynomials in these parameters and a certain number of new parameters with rational integer coefficients. (It is readily seen that the minimum number for the first equation is  $\frac{1}{2}(i^2 + 3i - 2)$ , and similarly for all.) The notation may be chosen so that the  $\alpha_p, \beta_q, \dots, \gamma_r$  in the solutions are expressed as the polynomials while the  $A'_p, B'_q, \dots, C'_r$  are expressed as the power products, so that

$$\begin{aligned} a_p A_p &= P_p, \quad b_q B_q = Q_q, \quad \dots, \quad c_r C_r = R_r, \\ (p &= 1, \dots, i; \quad q = 1, \dots, j; \quad \dots; \quad r = 1, \dots, k), \end{aligned}$$

where the  $P_p, Q_q, \dots, R_r$  are power products. This is a simple multiplicative system, and hence it is completely solvable by either method in § 1. The values of the indeterminates are obtained as power products. These values are substituted into the polynomial expressions for  $\alpha_p, \beta_q, \dots, \gamma_r$ . The method carries with it the proof that the solution of (3) thus obtained is the complete rational integer solution of (2).

3. *Generalization of § 2.*—It was shown elsewhere<sup>4</sup> that the method applied to solve a simple multiplicative diophantine system completely in rational integers can be extended immediately to obtain the complete solution of such a system in the integral elements of any ring in which the fundamental theorem of arithmetic holds. The passage from simple to compound systems in the ring of rational integers depends only on the existence of a G. C. D. for rational integers. Hence § 2 can be extended

immediately from the special Euclidean ring of rational integers to any Euclidean ring as defined in algebra.<sup>5</sup>

The simplest example is the ring of Gaussian integers. A system (2) of  $n$  equations in Gaussian integers is equivalent to a system of  $2n$  equations in rational integers. The complete rational integer solution of the  $2n$  equations is obtained from that of the original in Gaussian integers by equating reals and imaginaries. More generally, if a ring of algebraic integers of degree  $g$  is Euclidean, a system (2) of  $n$  equations in integers of the ring is equivalent to a system of  $gn$  equations in rational numbers (which can be made integral by a simple transformation of the equations); and the complete solution of the  $gn$  equations is obtained from that of the original  $n$  equations in integers of the ring by equating coefficients of like elements in the canonical (basis) representation of integers of the ring.

4. *Examples.*—Specimens of the simpler types of single equations will suffice to indicate the kind of result obtainable by the method. As noted in § 1, the number of parameters in a complete solution increases so rapidly with the degree and number of indeterminates in the system, that it is not possible to exhibit the actual solutions of any but the simplest systems in a reasonable space.

If  $c_1, \dots, c_n$  denote constant rational integers, the complete rational integer solution of

$$c_1x_1 + \dots + c_nx_n = 0$$

follows from Dickson's theorem.<sup>6</sup> For  $n = 3, 4$  the respective numbers of parameters are 3, 6.

If  $Q(x_1, \dots, x_n)$  is a quadratic form in  $x_1, \dots, x_n$  with rational integer coefficients, the complete rational integer solution of

$$Q(x_1, \dots, x_n) = Q(y_1, \dots, y_n)$$

is found from the reduction of this equation to the canonical form

$$a_1X_1^2 + \dots + a_nX_n^2 = a_1Y_1^2 + \dots + a_nY_n^2,$$

in which  $a_1, \dots, a_n$  are rational integers, and the  $X, Y$  are linear homogeneous functions of the  $x$ 's and  $y$ 's, respectively, with rational integer coefficients. The solution of the canonical equation follows in an obvious way from that

$$\sum_{j=1}^n a_j \mu_j v_j = 0,$$

and the solution of this in turn is given by that of

$$\sum_{j=1}^n w_j \mu_j v_j = 0,$$

in which the  $w, u, v$  are indeterminates. For  $n = 3$  and  $a_1 a_2 a_3 \neq 0$ , the solution of the canonical equation is by polynomials of degree 4 in 9 parameters, the coefficients in the polynomials are power products formed from the simultaneous resolution of the coefficients  $a_1, a_2, a_3$  into products of the respective forms  $k f_1 f_2 f_3 f_4 f_5 f_6 f_7 f_8$ ,  $k f_1 f_5 f_7 g_1 g_2 g_3 g_4 g_5$ ,  $k f_2 g_3 h_1 h_2$ , where all the letters denote rational integers.

The complete integer solution  $x, y, z, u, v, w$  of

$$xu^2 + yv^2 + zw^2 = 0$$

is by means of polynomials in 19 parameters with integer coefficients; the highest degree of a polynomial in the solution is 20 and the lowest 11.

In obtaining the complete integer solution of

$$xu^3 + yv^3 + zw^3 = 0,$$

the central detail is the complete integer solution of the system

$$abc = d^3, abe = f^3, ag = h^3,$$

for which 31 parameters are required; the respective degrees of  $a, b, c, d, e, f, g, h$  in the parameters are 43, 30, 14, 29, 71, 48, 68, 37.

<sup>1</sup> Bell, E. T., *Amer. Jour. Math.*, **55**, 50-66 (1933).

<sup>2</sup> Ward, M., *Ibid.*, **55**, 67-76 (1933).

<sup>3</sup> Dickson, L. E., *Modern Elementary Theory of Numbers*, Chicago, 194 (1939).

<sup>4</sup> Bell, E. T., *Trans. Amer. Math. Soc.*, **35**, 903-914 (1933).

<sup>5</sup> van der Waerden, B. L., *Moderne Algebra*, Berlin, **1**, § 19 (1937).

## ON LAGUERRE SERIES

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In an article to appear in the *Annals of Mathematics*, the author discussed certain convergence properties of the series:

$$g(s) = \sum_{n=1}^{\infty} a_n \exp(-\lambda_n s) G(\lambda_n s) \quad (1)$$

where (a)  $a_i$  was a complex constant,  $i = 1, 2, 3, \dots$

(b)  $0 < \lambda_1 < \lambda_2 < \lambda_3 < \dots < \lambda_n < \dots$

$$\lim_{n \rightarrow \infty} \lambda_n = \infty$$

(c)  $s = \sigma + it$ ,  $\sigma$  and  $t$  real,

and where the function  $G(z)$  satisfied certain conditions, in particular  $G(z)$  was bounded for  $R(z) \geq z_0 > 0$ . If  $G(z) \equiv 1$ , then the series (1) is a Dirichlet series of type  $\lambda_n$ ; the author named the series (1) a modified exponential series. As a special example the author dealt with such a modifying factor that (1) was a series of Hankel functions of the first kind of fixed order  $\nu$ .

The condition above on  $G(z)$  is very restrictive and prevents the modifying factor  $G(z)$  from being a polynomial in  $z$ . Consider now the series (1) when  $G(z)$  is replaced by the Laguerre polynomial of  $\nu$ th degree, i.e.,

$$f(s) = \sum_{n=1}^{\infty} a_n \exp(-\lambda_n s) L_{\nu}(2\lambda_n s) \quad (2)$$

where  $L_{\nu}(z)$  is the Laguerre polynomial of  $\nu$ th degree (fixed), and thus  $\exp\left(-\frac{z}{2}\right) L_{\nu}(z)$  the Laguerre function, and where conditions (a), (b) and (c) still hold.

In order to prove convergence properties of series (2) we shall establish a few lemmas.

LEMMA 1: There exists a constant  $K_1 > 0$  and a real number  $z_0$  such that

$$|L_{\nu}(z)| \geq K_1 > 0 \text{ for } R(z) \geq z_0.$$

*Proof:* Since  $L_{\nu}(z)$  is a polynomial of the  $\nu$ th degree with  $\nu$  roots,  $K_1$  and  $z_0$  (positive) can easily be selected satisfying the above.

LEMMA 2: For each  $\sigma_0 > 0$ , there exists a constant  $K_2$  and an integer  $N_1$  such that whenever  $x \geq \lambda_{N_1}$  and  $R(s) \geq \sigma_0 > 0$ , we have

$$\left| \exp\left(\frac{x(s_0 - s)}{2}\right) \frac{L_{\nu}(2xs)}{L_{\nu}(2xs_0)} \right| < K_2.$$

*Proof:* Given  $\sigma_0$ , we can choose  $N_1$  such that  $2\lambda_{N_1}\sigma_0 \geq z_0$  of Lemma 1, and thus for the range  $R(s) \geq \sigma_0$  we can divide by  $L_{\nu}(2xs)$ . Because of the exponential factor we have

$$\lim_{x \rightarrow \infty} \left| \exp\left(\frac{x(s_0 - s)}{2}\right) \frac{L_{\nu}(2xs)}{L_{\nu}(2xs_0)} \right| = 0.$$

Separate the interval  $(\lambda_{N_1}, \infty)$  into  $(\lambda_{N_1}, x_0)$  and  $(x_0, \infty)$  such that

$$\left| \exp\left(\frac{x(s_0 - s)}{2}\right) \frac{L_{\nu}(2xs)}{L_{\nu}(2xs_0)} \right| < A$$

for  $x \geq x_0$ . Then in the interval  $(\lambda_{N_1}, x_0)$  the function is continuous and has a maximum value, and thus the existence of a constant  $K_2$  and an integer  $N_1$  satisfying the conclusion of the lemma has been established.

LEMMA 3: For each  $\sigma_0 > 0$  there exists a constant  $K_3$  and an integer  $N_1$  such that for  $R(s) \geq \sigma_0$  and for  $x > \lambda_{N_1}$  we have

$$\left| \frac{\frac{\partial L_\nu(2xs_0)}{\partial x}}{L_\nu(2xs_0)} - \frac{\frac{\partial L_\nu(2xs)}{\partial x}}{L_\nu(2xs)} \right| < K_2 |s - s_0|.$$

*Proof:* Choosing  $N_1$  as in the preceding lemma, we write the above as the integral of its own derivative, and obtain

$$\left| \frac{\frac{\partial L_\nu(2xs_0)}{\partial x}}{L_\nu(2xs_0)} - \frac{\frac{\partial L_\nu(2xs)}{\partial x}}{L_\nu(2xs)} \right| = \left| \int_s^{s_0} 2x \left[ \frac{\frac{\partial^2 L_\nu(2xw)}{\partial w \partial x} L_\nu(2xw) - \frac{\partial L_\nu(2xw)}{\partial x} \frac{\partial L_\nu(2xw)}{\partial w}}{L_\nu^2(2xw)} \right] dw \right|.$$

Now because of the polynomial nature of  $L_\nu(z)$ , the integrand above is bounded for  $R(s) \geq \sigma_0$  and for  $x \geq \lambda_{N_1}$ , and thus can be made less than a constant  $K_3$  and we obtain, on integration, the desired conclusion.

LEMMA 4: For  $s_0 = \sigma_0 + it_0$ ,  $\sigma_0 > 0$ , there exists a constant  $N_1$  such that for  $R(s) \geq \sigma_0$  and  $x \geq \lambda_{N_1}$  we have

$$\left| \exp [\lambda_n(s_0 - s)] \frac{L_\nu(2\lambda_n s)}{L_\nu(2\lambda_n s_0)} - \exp [\lambda_{n+1}(s_0 - s)] \frac{L_\nu(2\lambda_{n+1} s)}{L_\nu(2\lambda_{n+1} s_0)} \right| \leq K_2(1 + K_3) \frac{2|s - s_0|}{R(s - s_0)} \left\{ \exp \left[ \frac{\lambda_n R(s_0 - s)}{2} \right] - \exp \left[ \frac{\lambda_{n+1} R(s_0 - s)}{2} \right] \right\}.$$

*Proof:* Writing the function as the integral of its own derivative we have

$$\begin{aligned} \left| \exp [\lambda_n(s_0 - s)] \frac{L_\nu(2\lambda_n s)}{L_\nu(2\lambda_n s_0)} - \exp [\lambda_{n+1}(s_0 - s)] \frac{L_\nu(2\lambda_{n+1} s)}{L_\nu(2\lambda_{n+1} s_0)} \right| &= \\ \left| \int_{\lambda_n}^{\lambda_{n+1}} \left\{ (s_0 - s) \exp [x(s_0 - s)] \frac{L_\nu(2xs)}{L_\nu(2xs_0)} + \right. \right. \\ &\quad \left. \left. \exp [x(s_0 - s)] \frac{L_\nu(2xs)}{L_\nu(2xs_0)} \left[ \frac{\frac{\partial L_\nu(2xs)}{\partial x}}{L_\nu(2xs)} - \frac{\frac{\partial L_\nu(2xs_0)}{\partial x}}{L_\nu(2xs_0)} \right] \right\} dx \right| \leq \\ \int_{\lambda_n}^{\lambda_{n+1}} \left| (s_0 - s) K_2 \exp \left[ \frac{x(s_0 - s)}{2} \right] + \exp \left[ \frac{x(s_0 - s)}{2} \right] K_2 K_3 (s_0 - s) \right| dx &\leq \\ K_2(1 + K_3) |s_0 - s| \int_{\lambda_n}^{\lambda_{n+1}} \left| \exp \left[ \frac{x(s_0 - s)}{2} \right] \right| dx &\leq \\ \frac{K_2(1 + K_3) 2|s_0 - s|}{R(s - s_0)} \left\{ \exp \left[ \frac{\lambda_n R(s_0 - s)}{2} \right] - \exp \left[ \frac{\lambda_{n+1} R(s_0 - s)}{2} \right] \right\}. \end{aligned}$$



LEMMA 5: *Abel's Lemma on Partial Summation.*—We have identically:

$$\sum_{n=p}^{n=q} U_n V_n = \sum_{n=p}^{n=q-1} \left[ (V_n - V_{n+1}) \sum_{m=p}^{m=n} U_m \right] + V_q \sum_{m=p}^{m=q} U_m.$$

THEOREM 1: If  $f(s) = \sum_{n=1}^{\infty} a_n \exp(-\lambda_n s) L_\nu(2\lambda_n s)$  is convergent for  $s = s_0 = \sigma_0 + i\tau_0$  where  $\sigma_0 > 0$ , then it is uniformly convergent throughout the angular sector defined by  $|\arg(s - s_0)| \leq \psi < \frac{\pi}{2}$ .

*Proof:* Since  $\sigma_0 > 0$ , we can select the integer  $N_1$  of Lemmas 2, 3 and 4. In the infinite angular sector  $|\arg(s - s_0)| \leq \psi < \frac{\pi}{2}$ , the finite summation  $\sum_{n=1}^k a_n \exp(-\lambda_n s) L_\nu(2\lambda_n s)$  gives no trouble, since each term is bounded.

Thus we need only show that given  $\epsilon_1 > 0$ , we can find an integer  $n_1$  such that for  $q \geq p \geq n_1$  we have

$$\left| \sum_{n=p}^{n=q} a_n \exp(-\lambda_n s) L_\nu(2\lambda_n s) \right| < \epsilon_1 \quad (3)$$

for all  $s$  in the angular sector.

Applying Abel's lemma we get

$$\begin{aligned} \left| \sum_{n=p}^{n=q} a_n \exp(-\lambda_n s) L_\nu(2\lambda_n s) \right| &\leq \\ \sum_{n=p}^{n=q-1} &\left| \left\{ \exp[\lambda_n(s_0 - s)] \frac{L_\nu(2\lambda_n s)}{L_\nu(2\lambda_n s_0)} - \exp[\lambda_{n+1}(s_0 - s)] \frac{L_\nu(2\lambda_{n+1} s)}{L_\nu(2\lambda_{n+1} s_0)} \right\} \right| \cdot \\ &\cdot \left| \sum_{m=p}^{m=n} a_m \exp[-\lambda_m s_0] L_\nu(2\lambda_m s_0) \right| + \\ &\left| \exp[\lambda_q(s_0 - s)] \frac{L_\nu(2\lambda_q s)}{L_\nu(2\lambda_q s_0)} \right| \cdot \left| \sum_{m=p}^{m=q} a_m \exp(-\lambda_m s_0) L_\nu(2\lambda_m s_0) \right|. \end{aligned} \quad (4)$$

Now given  $\sigma_0$ ,  $\epsilon_1$  and  $\psi$  (each a constant) we can select  $\epsilon = \epsilon(\sigma_0, \epsilon_1, \psi)$ ; the exact functional form will be specified later. Given this  $\epsilon$ , we can select  $N_2$  such that

$$\left| \sum_{m=p}^{m=q} a_m \exp[-\lambda_m s_0] L_\nu(2\lambda_m s_0) \right| < \epsilon \quad (5)$$

for  $q \geq p \geq N_2$ .

Substituting (5) into (4) and using the result of Lemma 4 we obtain, where  $n \geq n_1 = \max[N_1, N_2]$ ,

$$\begin{aligned}
& \left| \sum_{n=p}^{n=q} a_n \exp(-\lambda_n s) L_\nu(2\lambda_n s) \right| \leq \\
& \epsilon K_2(1 + K_3) \frac{2|s - s_0|}{R(s - s_0)} \sum_{n=p}^{n=q-1} \left\{ \exp \left[ \frac{\lambda_n R(s_0 - s)}{2} \right] - \exp \left[ \frac{\lambda_{n+1} R(s_0 - s)}{2} \right] \right\} \\
& \quad \quad \quad (6) \\
& \quad \quad \quad + \epsilon K_2 \exp \left[ \frac{\lambda_q R(s_0 - s)}{2} \right] \leq \\
& \quad \quad \quad \epsilon K_2(1 + K_3) \frac{2|s - s_0|}{R(s - s_0)} \exp \left[ \frac{\lambda_q R(s_0 - s)}{2} \right] \leq \\
& \quad \quad \quad \epsilon K_2(1 + K_3) \cdot 2 \cdot \sec \psi
\end{aligned}$$

since  $\frac{|s - s_0|}{R(s - s_0)} = \sec \{ |a_m(s - s_0)| \} \leq \sec \psi$ .

Thus by choosing  $\epsilon = \frac{\epsilon_1}{2K_2(\sigma_0)[1 + K_3(\sigma_0)] \sec \psi}$  we see that (3) is satisfied and the uniform convergence of the Laguerre series in the angular sector is established.

**THEOREM 2:** If the Laguerre series is convergent for  $s = s_0 = \sigma_0 + it_0$  where  $\sigma_0 > 0$ , then it is convergent in the half plane  $R(s) > \sigma_0$ .

*Proof:* This theorem is implicit in the more general theorem 1.

We can thus define an *abscissa of convergence*  $\alpha$  and a *line of convergence*  $R(s) = \alpha$  for the Laguerre series (2) by taking a Dedekind cut of the non-negative real numbers. Theorem 3, which follows, shows that we can define an *abscissa of absolute convergence*  $\beta$  and a *line of absolute convergence*  $R(s) = \beta$ . Notice, as is also the case with Dirichlet series and the modified exponential series (1), that convergence is proved only for  $R(s) > \alpha$  while absolute convergence will be proved for  $R(s) \geq \beta$ .

**THEOREM 3:** If  $f(s) = \sum_{n=1}^{\infty} a_n \exp(-\lambda_n s) L_\nu(2\lambda_n s)$  is absolutely convergent for  $s = s_1 = \sigma_1 + it_1$ , where  $\sigma_1 > 0$ , then it is absolutely convergent in the half plane  $R(s) \geq \sigma_1$ .

*Proof:* We shall show that for  $R(s) \geq \sigma_1$ , given  $\epsilon_2 > 0$ , we can find an integer  $n_2$  such that for  $q \geq p \geq n_2$  we have

$$\sum_{n=p}^{n=q} |a_n| \exp(-\lambda_n \sigma) |L_\nu(2\lambda_n s)| < \epsilon_2.$$

By hypothesis, given  $\epsilon > 0$ , there exists an integer  $N_2$  such that for  $q \geq p \geq N_2$  we have

$$\sum_{n=p}^{n=q} |a_n| \exp(-\lambda_n \sigma_1) |L_\nu(2\lambda_n s_1)| < \epsilon.$$

Take  $n_2 = \max [N_2, N_1]$  where  $N_1$  is given by Lemma 2. We have

$$\sum_{n=p}^{n=q} |a_n| \exp(-\lambda_n \sigma) |L_\nu(2\lambda_n s)| \leq \sum_{n=p}^{n=q} |a_n| \exp(-\lambda_n \sigma_1) |L_\nu(2\lambda_n s_1)| \left| \frac{L_\nu(2\lambda_n s)}{L_\nu(2\lambda_n s_1)} \right| \exp[(\sigma_1 - \sigma)\lambda_n] \leq \epsilon K_2$$

where  $K_2$  is given by Lemma 2, and thus by proper choice of  $\epsilon$  the desired inequality is obtained.

## EQUILONG AND CONFORMAL TRANSFORMATIONS OF PERIOD TWO

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1. *Introduction.*—Our main problem is to find all equilong transformations of period two. We find that in equilong geometry the set of all equilong transformations of period two may be classified into *three* distinct types:  $(T_1)$  equilong involutions,  $(T_2)$   $K$  symmetries and  $(T_3)$   $D$  inversions. This is in contrast with the conformal theory where Kasner has proved that the set of all conformal transformations of period two consists of *two* distinct types:  $(\mathfrak{T}_1)$  conformal involutions, and  $(\mathfrak{T}_2)$  conformal symmetries (the Schwarzian reflections).

*We thus have five distinct types of transformations of period two: three equilong and two conformal.*

The conformal types (and the groups generated by them) were discussed by Kasner.<sup>1</sup> In this and later papers, we are going to discuss the analogous situations in equilong geometry.

We shall consider only those equilong transformations of the plane which convert the positive  $y_1$ -axis (with hessian or equilong coördinates  $(0, 0)$ ) into itself and are regular at the positive  $y_1$ -axis. Such regular equilong transformations are expressed by power series of the two forms

$$Z = \alpha_1 z + \alpha_2 z^2 + \dots, \alpha_1 \alpha_1 \neq 0, \quad (1.1)$$

$$\bar{Z} = \alpha_1' \bar{z} + \alpha_2' \bar{z}^2 + \dots, \alpha_1' \bar{\alpha}_1' \neq 0, \quad (1.2)$$

where  $\bar{z} = x - jy$  is the conjugate of the dual variable  $z = x + jy$  with  $j^2 = 0$  and  $(x, y)$  the hessian or equilong coördinates of a line, and the coefficients are arbitrary dual numbers of the form  $a + jb$ . Separating these equations into the two real forms, we find

$$\begin{aligned} X &= a_1x + a_2x^2 + \dots, \\ Y &= y(a_1 + 2a_2x + \dots) + (b_1x + b_2x^2 + \dots), \end{aligned} \quad (2.1)$$

$$\begin{aligned} X &= a_1'x + a_2'x^2 + \dots, \\ Y &= -y(a_1' + 2a_2'x + \dots) - (b_1'x + b_2'x^2 + \dots), \end{aligned} \quad (2.2)$$

The *direct* equilong transformations (1.1) or (2.1) form a continuous infinite group  $G$ . The *reverse* equilong transformations (1.2) or (2.2) do not form a group. However, if we add this set to our group  $G$ , we obtain a mixed group  $G'$ .

We determine all regular equilong transformations of period two. In the direct type  $Z = f(z)$  the functional equation is  $f[f(z)] \equiv z$ , that is,  $f^2 = 1$ ; and in the reverse type  $\bar{Z} = f(z)$  the functional equation is  $\bar{f}[f(z)] \equiv z$ , that is,  $\bar{f}f = 1$  where  $\bar{f}$  denotes the series whose coefficients are the conjugates of those of the series  $f$ . Thus we are to solve two types of functional equations. The first type  $f^2 = 1$  yields as solutions *a single set* ( $T_1$ ) of equilong involutions (besides the identity). On the other hand, the second type  $\bar{f}f = 1$  admits as solutions *two equilongly distinct sets* ( $T_2$ ) of  $K$  symmetries and ( $T_3$ ) of  $D$  inversions.

To find the regular conformal transformations of period two, the analogous functional equations  $f^2 = 1$  and  $\bar{f}f = 1$  have to be solved. The first type  $f^2 = 1$  yields as solutions *a single set* ( $\mathfrak{T}_1$ ) of conformal involutions (besides the identity). The second type  $\bar{f}f = 1$  admits as solutions *a single set* ( $\mathfrak{T}_2$ ) of conformal symmetries (Schwarzian reflections).

It can be shown that ( $T_1$ ) any equilong involution can be reduced equilongly to the simple form  $Z = -z$  (symmetry in the positive  $y_1$ -axis), ( $T_2$ ) any  $K$  symmetry can be changed equilongly to the simple form  $Z = \bar{z}$  (symmetry in the origin accompanied by reversal of orientation) and ( $T_3$ ) any  $D$  inversion can be reduced equilongly to the simple form  $Z = -\bar{z}$  (symmetry in the  $x_1$ -axis accompanied by reversal of orientation). These simple symmetries are to be regarded as transformations between the lines of the plane.

In the conformal theory, we recall that ( $\mathfrak{T}_1$ ) any conformal involution can be changed conformally to the simple form  $Z_1 = -z_1$  (symmetry in the origin), and ( $\mathfrak{T}_2$ ) any conformal symmetry may be reduced conformally to the simple form  $Z_1 = \bar{z}_1$  (symmetry in the  $x_1$ -axis), where  $\bar{z}_1 = x_1 - iy_1$  is the conjugate of the complex variable  $z_1 = x_1 + iy_1$  with  $i^2 = -1$  and  $(x_1, y_1)$  the cartesian coördinates of a point. These symmetries are considered to be transformations between the points of the plane.

2. *The Discussion of ( $T_1$ ) Equilong Involutions.*—To obtain the equations of any equilong involution in the implicit form, it is convenient to rewrite any direct equilong transformation (1.1) in another form. Let  $\lambda = Z + z$ ,  $\mu = Z - z$  so that  $Z = \frac{1}{2}(\lambda + \mu)$ ,  $z = \frac{1}{2}(\lambda - \mu)$ . Substituting these results into (1.1), we find

$$\frac{1}{2}(1 - \alpha_1)\lambda + \frac{1}{2}(1 + \alpha_1)\mu = \alpha_2/\lambda^2(\lambda - \mu)^2 + \dots \quad (3)$$

If  $\alpha_1 \neq -1 + jb$  ( $b$  real), we can solve this for  $\mu$  as a power series in  $\lambda$ . On the other hand, if  $\alpha_1 \neq 1 + jb$  ( $b$  real), we can solve this for  $\lambda$  as a power series in  $\mu$ . Hence in all cases, any direct equilong transformation can be written in either or both of the two forms

$$Z - z = \beta_1(Z + z) + \beta_2(Z + z)^2 + \dots, \quad (4.1)$$

$$Z + z = \gamma_1(Z - z) + \gamma_2(Z - z)^2 + \dots \quad (4.2)$$

Thus the group  $G$  of all direct equilong transformations has been subdivided into two sets (4.1) and (4.2) which are *not* mutually exclusive.

Since any direct equilong transformation, defined implicitly by  $F(Z, z) = 0$ , is an involution if and only if it is symmetric in the two variables, that is,  $F(Z, z) \equiv F(z, Z)$ , we obtain from (4.1) and (4.2) the following result.

**THEOREM 1.** *Any equilong involution is given in the implicit form by*

$$Z + z = \alpha_2(Z - z)^2 + \alpha_4(Z - z)^4 + \dots, \quad (5)$$

where the  $\alpha_{2n}$  are arbitrary dual numbers. In the real implicit form, any equilong involution is

$$X + x = a_2(X - x)^2 + a_4(X - x)^4 + \dots,$$

$$Y + y = (Y - y)[2a_2(X - x) + 4a_4(X - x)^3 + \dots] \\ + [b_2(X - x)^2 + b_4(X - x)^4 + \dots]. \quad (6)$$

Any equilong involution in the explicit dual variable form is

$$Z = -z + \gamma_2 z^2 - \gamma_2^2 z^3 + \gamma_4 z^4 + (-3\gamma_2\gamma_4 + 2\gamma_2^4)z^5 + \dots, \quad (7)$$

where the  $\gamma_{2n}$  are arbitrary dual numbers. Finally in the real explicit form any equilong involution is

$$X = -x + c_2 x^2 - c_2^2 x^3 + c_4 x^4 + (-3c_2 c_4 + 2c_2^4)x^5 + \dots,$$

$$Y = y[-1 + 2c_2 x - 3c_2^2 x^2 + 4c_4 x^3 + 5(-3c_2 c_4 + 2c_2^4)x^4 + \dots] + \\ [d_2 x^2 - 2c_2 d_2 x^3 + d_4 x^4 + (-3c_2 d_4 - 3c_4 d_2 + 8c_2^3 d_2)x^5 + \dots]. \quad (8)$$

3. *The Discussion of ( $T_2$ )  $K$  Symmetries.*<sup>2</sup>—To find the equations of any  $K$  symmetry and of any  $D$  inversion, it is convenient to rewrite any reverse equilong transformation (1.2) in another form. By an argument very similar to the one given at the beginning of section 2, we find in all cases that any reverse equilong transformation can be written in either or both of the two forms

$$\bar{Z} - z = \beta_1(\bar{Z} + z) + \beta_2(\bar{Z} + z)^2 + \dots, \quad (9.1)$$

$$\bar{Z} + z = \gamma_1(\bar{Z} - z) + \gamma_2(\bar{Z} - z)^2 + \dots \quad (9.2)$$

The set of all reverse equilog transformations has been subdivided into two sets (9.1) and (9.2) which are *not* mutually exclusive.

A *K symmetry* is any reverse equilog transformation of period two which is of the form (9.1). On the other hand, any *D inversion* is any reverse equilog transformation of period two which is of the form (9.2).

Since the inverse of any *K symmetry* is itself, the solution for  $\bar{Z}$  of the equation  $\bar{Z} - z = f(\bar{Z} + z)$  must be identical with the solution for  $z$  of the conjugate equation  $\bar{z} - Z = -\bar{f}(\bar{z} + Z)$ . Therefore  $\beta_n = -\bar{\beta}_n$  for all  $n$ . Thus the power series expansion in  $\bar{Z} + z$  has as coefficients only *pure dual numbers*. Hence

**THEOREM 2.** *Any K symmetry is given in the implicit form by the equation*

$$\bar{Z} - z = j[b_1(\bar{Z} + z) + b_2(\bar{Z} + z)^2 + \dots], \quad (10)$$

where the  $b_n$  are real numbers. In the real explicit form, any *K symmetry* is

$$X = x, Y = -y + 2(d_1x + d_2x^2 + \dots). \quad (11)$$

In the explicit dual variable form, any *K symmetry* may be written as

$$\bar{Z} = z - 2j(d_1z + d_2z^2 + \dots). \quad (12)$$

4. *The Discussion of ( $T_3$ ) D Inversions.*—Observing that the inverse of any *D inversion* is itself, the solution for  $\bar{Z}$  of the equation  $\bar{Z} + z = f(\bar{Z} - z)$  must be identical with the solution for  $\bar{z}$  of the conjugate equation  $\bar{z} + Z = \bar{f}[-(\bar{z} - Z)]$ . Therefore  $\gamma_{2n-1} = -\bar{\gamma}_{2n-1}$  and  $\gamma_{2n} = \bar{\gamma}_{2n}$  for all  $n$ . Thus in the power series expansion in  $(\bar{Z} - z)$ , the odd coefficients must be *pure dual numbers* and the even coefficients must be real numbers. Hence

**THEOREM 3.** *Any D inversion is given in the implicit form by the equation*

$$\begin{aligned} \bar{Z} + z = [a_2(\bar{Z} - z)^2 + a_4(\bar{Z} - z)^4 + \dots] \\ + j[b_1(\bar{Z} - z) + b_3(\bar{Z} - z)^3 + \dots], \end{aligned} \quad (13)$$

where the  $a_{2n}$  and the  $b_{2n-1}$  are real numbers. In the real implicit form, any *D inversion* may be written as

$$\begin{aligned} X + x = a_2(X - x)^2 + a_4(X - x)^4 + \dots, \\ Y - y = (Y + y)[2a_2(X - x) + 4a_4(X - x)^3 + \dots] \\ - [b_1(X - x) + b_3(X - x)^3 + \dots]. \end{aligned} \quad (14)$$

Finally in the real explicit form any *D inversion* is

$$\begin{aligned} X = -x + c_2x^2 - c_3^2x^3 + c_4x^4 + (-3c_2c_4 + 2c_3^4)x^5 + \dots, \\ Y = -y[-1 + 2c_2x - 3c_3^2x^2 + 4c_4x^3 + 5(-3c_2c_4 + 2c_3^4)x^4 + \dots] - \\ [d_1x - 3/2c_2d_1x^2 + d_2x^2 + 5/2(-c_2d_2 - c_4d_1 + 2c_3^2d_1)x^4 + d_3x^3 + \dots]. \end{aligned} \quad (15)$$



5. *The Reduction of the  $(T_1)$  Equilong Involutions,  $(T_2)$   $K$  Symmetries and  $(T_3)$   $D$  Inversions to Canonical Forms.*—In the first place, it may be shown that there exists formally at least one real function  $\Phi(x) = \sum c_n x^n$  ( $c_1 \neq 0$ ) such that  $\Phi(X) + \Phi(x) \equiv 0$  where  $X$  is any real function of period two, given implicitly by the first of equations (6) or (14). It results that all the even coefficients  $c_{2n}$  are determined as linear functions of the odd coefficients  $c_{2n-1}$  such that the coefficients of these linear functions are polynomials in the  $a_{2n}$ . Thus there exist many such functions  $\Phi(x)$ .

Under the direct equilong transformation  $X = \Phi(x)$ ,  $Y = y\Phi_x(x)$ , our equilong involution (6) becomes  $X = -x$ ,  $Y = -y + 2(d_2x^2 + d_4x^4 + \dots)$ . The transform of this under the direct equilong transformation  $X = x$ ,  $Y = y - (d_2x^2 + d_4x^4 + \dots)$  is the equilong involution  $X = -x$ ,  $Y = -y$ .

Under the direct equilong transformation  $X = x$ ,  $Y = y - (d_1x + d_3x^3 + \dots)$ , the  $K$  symmetry (11) is reduced to the  $K$  symmetry  $X = x$ ,  $Y = -y$ .

Under the direct equilong transformation  $X = \Phi(x)$ ,  $Y = y\Phi_x(x)$ , our  $D$  inversion (14) becomes  $X = -x$ ,  $Y = y + 2(d_1x + d_3x^3 + \dots)$ . The transform of this under the direct equilong transformation  $X = x$ ,  $Y = y + (d_1x + d_3x^3 + \dots)$  is the  $D$  inversion  $X = -x$ ,  $Y = y$ . Therefore, we discover the following result.

**THEOREM 4.** *Any equilong involution can be reduced equilongly to the symmetry through the positive  $y_1$ -axis  $Z = -z$ . Any  $K$  symmetry may be changed equilongly to the symmetry through the origin accompanied by reversal of orientation  $Z = z$ . Any  $D$  inversion may be reduced equilongly to the symmetry through the  $x_1$ -axis accompanied by reversal of orientation  $Z = -z$ .*

It may be shown without any difficulty that there do not exist any direct or reverse equilong transformations which will carry any one of the three canonical forms  $(T_1)$   $X = -x$ ,  $Y = -y$ ,  $(T_2)$   $X = x$ ,  $Y = -y$ ,  $(T_3)$   $X = -x$ ,  $Y = y$  into the other. From this, we may derive the following proposition.

**THEOREM 5.** *All the equilong transformations of period two consist of the three equilongly mutually exclusive sets:  $(T_1)$  equilong involutions,  $(T_2)$   $K$  symmetries and  $(T_3)$   $D$  inversions.*

We note that  $D$  inversion is equilongly equivalent to Laguerre inversion, whereas  $K$  symmetry is equivalent to  $K$  inversion (with respect to a circle). Any  $K$  symmetry is the product of three  $D$  inversions. But no  $D$  inversion can be factored into any number of  $K$  symmetries.

In the conformal theory, any conformal symmetry is conformally equivalent to Moebius inversion. Thus we have *three* distinct types of inversion in circle geometry:  $(C_1)$  Moebius inversion,  $(C_2)$  Laguerre inversion and  $(C_3)$   $K$  inversion.

<sup>1</sup> Kasner, "Infinite Groups Generated by Conformal Transformations of Period Two (Involution and Symmetries)," *Am. Jour. Math.*, 38, 177-184 (1916); Kasner, "Conformal Geometry," *Proc. Int. Cong. Math.*, 2, 81 (1912).

<sup>2</sup> Kasner, "Equilong Symmetry with Respect to Any Curve," *Proc. Nat. Acad. Sci.*, **26**, 287-291 (1940).

<sup>3</sup> Kasner, "Geometry of Conformal Symmetry (Schwarzian Reflection)," *Ann. Math.*, **38**, 873-879 (1937); Pfeiffer, "On the Conformal Geometry of Analytic Arcs," *Am. Jour. Math.*, **37**, 395-430 (1915); Comenetz, "Conformal Geometry on a Surface," *Ann. Math.*, **39**, 863-871 (1938).

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## RADIOACTIVE CARBON AS AN INDICATOR OF CARBON DIOXIDE REDUCTION. IV. THE SYNTHESIS OF ACETIC ACID FROM CARBON DIOXIDE BY *CLOSTRIDIUM ACIDI-URICI*

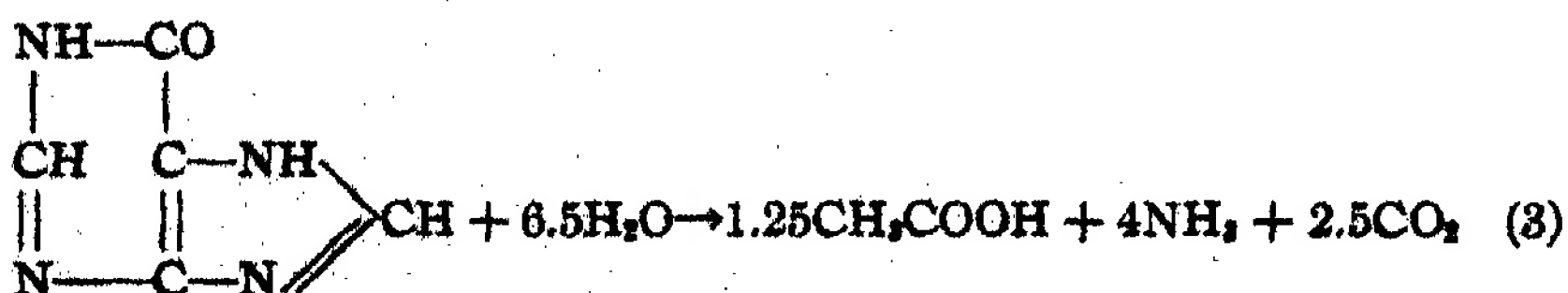
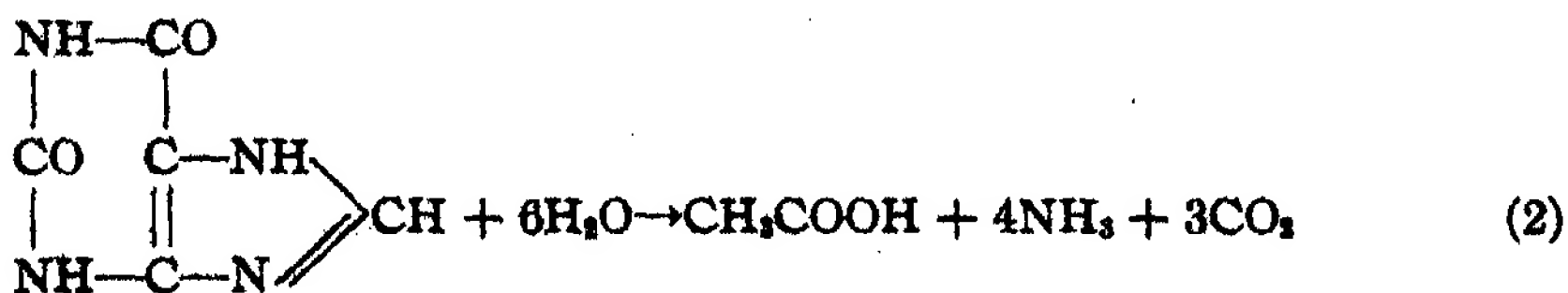
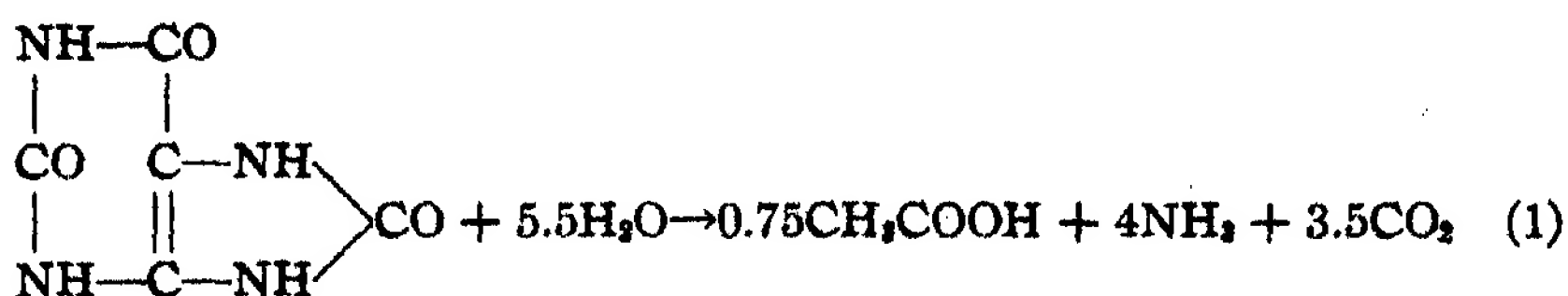
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*Clostridium acidi-urici* is a little-known organism\* capable of rapidly fermenting uric acid, xanthine, hypoxanthine and other purines under strictly anaerobic conditions. Besides cell materials, only three products are formed in considerable quantities in these fermentations, namely, ammonia, carbon dioxide and acetic acid. These substances account for about 98% of the nitrogen and 95% of the carbon of the purines decomposed.

The relative quantities of the fermentation products depend, of course, upon the particular compound used. Uric acid, xanthine and hypoxanthine, for example, are fermented approximately according to the following equations:



Actually the observed yields of acetic acid are a little lower than indicated by these equations. With uric acid the observed yields in moles of acetic acid per mole of purine fermented have been 0.6–0.7, with xanthine 0.9–1.0 and with hypoxanthine 1.1–1.25.

Now the yields of acetic acid obtained from uric acid and xanthine are in no way remarkable since these quantities might well be derived from the central  $C_3$  chain of these purines. With hypoxanthine, however, the quantity of acetic acid is greater than could be obtained directly from this source. To explain the formation of more than one mole of acetic acid per mole of hypoxanthine it must be postulated that either (1) an intra- or intermolecular condensation involving the  $C_3$  chains occurs followed by a splitting into the required number of  $C_2$  fragments, or (2) part or all of the acetic acid is built up from the carbon dioxide or other  $C_1$  fragments of the purine.

Using radioactive carbon ( $C^{11}$ ) as an indicator it has been possible to test the latter alternative and to show that acetic acid is formed by the reduction of carbon dioxide.

For the experiments reported below, suspensions of *Cl. acidi-urici*, strain 9a, were used. The organisms were grown in a medium containing 0.3–0.4% uric acid, 0.1% yeast autolyzate, mineral salts and sodium sulfide or thioglycollate as a reducing agent. The reaction was adjusted to pH 7.4. After 18–24 hours' incubation at 35°C. under strictly anaerobic conditions, the cells were centrifuged, washed and resuspended in  $M/10$  phosphate buffer pH 7 containing 0.015%  $Na_2S \cdot 9H_2O$ . Cells derived from 250–1000 cc. of medium were used in each experimental vessel. A brief description of the mode of preparation and use of  $C^{11}$  (21 minutes half-life) is given elsewhere.<sup>5</sup>

*Experiment 1.*—The object of the first experiment was to see whether radioactive acetic acid is formed during fermentations of uric acid, guanine and hypoxanthine carried out in the presence of radioactive carbon dioxide. Three vessels were used, each containing 5 cc. of cell suspension plus the substances indicated in column 2 of table 1. Glycine was added to vessels 1 and 2 because it has been found to eliminate an induction period in the decomposition of these compounds. The cells were mixed with their substrates just before adding the radioactive carbon dioxide ( $C^*O_2$ ). The suspensions were then shaken in a  $N_2-C^*O_2$  atmosphere at 37°C.

Following the incubation period the contents of each vessel were treated with 0.5 g.  $NaHCO_3$ , 0.5 cc. 50% acetic acid and sufficient sulfuric acid to bring the reaction to pH 1; the mixture was then boiled briefly to remove all residual radioactive carbon dioxide. The volatile acids were then removed by steam distillation, neutralized and tested for radioactivity with a Geiger counter.

It can be seen in table 1 that the volatile acids derived from all three

compounds were highly radioactive. The non-volatile fractions were also active. The non-volatile material derived from the hypoxanthine cells contained only about 5% of the total activity; a considerable part of this can be attributed to an incomplete removal of volatile acids. The non-

TABLE 1

DISTRIBUTION OF RADIOACTIVE CARBON IN PRODUCTS OF PURINE FERMENTATION. (ACTIVITIES ARE EXPRESSED IN ARBITRARY UNITS; ALL VALUES ARE CORRECTED FOR DECAY AND ARE DIRECTLY COMPARABLE)

VESSEL	SUBSTRATE	INCUBATION TIME, MINUTES	PURINE DECOMPOSED MG.	RADIOACTIVITY VOLATILE ACIDS	RADIOACTIVITY NON-VOLATILE FRACTIONS
1	30 Mg. Na urate 1 Mg. glycine	15	23	2.3	1.97
2	20 Mg. guanine 1 Mg. glycine	39	9.6	1.35	...
3	10 Mg. hypo- xanthine	79	ca3	7.9	0.45

volatile activity from the uric acid cells was much larger, being sufficient to account for 46% of the total reduced C\*. Not more than about 10% of this 46%—5% of the total—can be attributed to residual volatile acids. It must be concluded that a considerable quantity of some truly non-volatile material has been formed by a reduction of carbon dioxide.

In order to positively identify the volatile acid fraction, it was subjected to Duclaux distillation; the volatile acid from the uric acid culture was used. Five 20-cc. fractions of distillate were collected from a total initial volume of 110 cc. In table 1 the activities of each fraction, expressed in per cent of the activity in 100 cc. of distillate, are compared with similar data obtained by distillation and titration of pure acetic acid solution. The agreement is sufficiently close to leave no doubt that the volatile acid produced from uric acid is very largely if not entirely acetic acid.

TABLE 2

IDENTIFICATION OF VOLATILE ACID FRACTION BY DUCLAUX DISTILLATION

VOLUME OF DISTILLATE	UNKNOWN % OF ACTIVITY	ACETIC ACID % OF TITRATION
20	14.3	15.7
40	30.9	32.7
60	50.8	51.3
80	73.8	72.7
100	100	100

*Experiment 2.*—A second experiment was undertaken to find out whether (1) the large quantity of radioactive non-volatile material formed from uric acid is dependent upon the presence of glycine; (2) the non-volatile fraction is soluble or is associated with the insoluble cell materials; (3) the radioactive carbon from carbon dioxide is in the methyl group of the acetic acid.

The procedure was essentially the same as in the previous experiment. Two vessels were used, each containing 5 cc. of cell suspension. In addition, one contained about 30 mg. of sodium urate and 1 mg. glycine; the other vessel contained urate only. Incubation was at 40°C. for 17 minutes and 65 minutes, respectively. In both vessels 22.8 mg. of uric acid were decomposed.

The distribution of radioactive carbon in various fractions of the fermented media is given in table 3. The fractionation of the contents of vessel 1 (containing glycine) was most complete. Immediately after stopping the incubation the cell suspension was centrifuged sharply to separate the cells from the soluble constituents. Volatile acid and non-volatile fractions were then prepared from both cells and supernatant solution. The suspension of vessel 2 (without glycine) was not centrifuged prior to separating the volatile and non-volatile fractions.

TABLE 3

DISTRIBUTION OF RADIOACTIVE CARBON IN PRODUCTS OF URIC ACID FERMENTATION. (ACTIVITIES ARE EXPRESSED IN ARBITRARY UNITS; ALL VALUES ARE CORRECTED FOR DECAY AND ARE DIRECTLY COMPARABLE)

FRACTION	VOLATILE	NON-VOLATILE	TOTAL	<u>NON-VOLATILE</u> TOTAL
Vessel 1 (glycine)				
Supernatant solution	17.4	8.5	25.9	0.33
Cells	1.9	4.7	6.6	0.71
Total	19.3	13.2	32.5	0.41
Vessel 2 (no glycine)				
Total	22.0	11.5	33.5	0.34

Table 3 shows that glycine does not markedly affect the total quantities or ratios of volatile and non-volatile activities. Non-volatile activity is present in both the cell fraction and the supernatant liquid. But although the total non-volatile activity is about twice as great in the liquid (9 cc.) as in the cell fraction (1 cc.), the concentration is much higher in the cells. This suggests that at least a considerable part of the radioactive non-volatile material is a cellular constituent. Further experiments would be required to elucidate the nature of this material.

The acetic acid was decarboxylated in order to find out if carbon from carbon dioxide was present in the methyl group. The volatile acid from vessel 1 was neutralized with Ba(OH)<sub>2</sub>, evaporated to dryness, finely powdered and heated at 450–500°C. for 20 minutes in a stream of oxygen-free nitrogen. The procedure used by Ardagh, *et al.*,<sup>1</sup> was followed. The resulting acetone was trapped in ice-cold 1*N* KOH and converted into iodoform by oxidation with iodine. The equations for the decarboxylation and oxidation reactions are:





The iodoform, separated by centrifugation and ether extraction, was found to be radioactive, the activity being sufficient to account for approximately 18% of the total activity of the initial acetic acid. Since only one molecule of iodoform is produced from two molecules of acetic acid, the observed activity is sufficient to account for 70% of the theoretical yield on the assumption that the C\* is distributed equally between the methyl and carboxyl groups. This calculated yield is a lower limit since the experiment was not carried out in such a way as to give a quantitative recovery of iodoform. Nevertheless the data constitute proof that an appreciable part of the reduced carbon is present in the methyl group.

*Experiment 3.*—This experiment was designed to give further information on the distribution of radioactive carbon between the methyl and carboxyl groups of acetic acid. The procedure was essentially the same as employed in the latter part of experiment 2 except that more care was exercised in the manipulations and, following decarboxylation, the activity of the residual  $\text{BaCO}_3$  as well as of the  $\text{CHI}_3$  was measured.

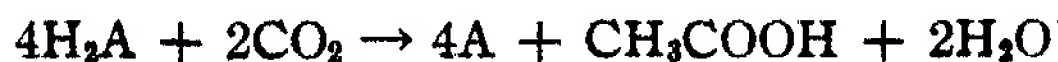
In this experiment the decarboxylation was allowed to proceed at 450–500°C. for 50 minutes. The yield of acetone was determined, following the iodoform reaction, by back titration with thiosulfate. There was obtained 68.5% of the theoretical yield. The iodoform was separated by centrifugation and exhaustive ether extraction, care being taken to have the aqueous phase alkaline to prevent contamination with acetic acid. The carbon dioxide resulting from decarboxylation was obtained by acidifying the residual  $\text{BaCO}_3$ , heating and collecting the evolved gas in alkali. The radioactivity of an aliquot of the alkali solution was determined.

The result of the experiment was the recovery of 23% of the activity of the acetic acid as iodoform derived from the methyl group and 11.3% as carbon dioxide derived from the carboxyl group. When these figures are corrected for the yield of acetone, they become 33.6 and 16.5%, respectively. From equations 4 and 5 it is evident that one of the two acetate radicals in the decomposed barium acetate is regenerated intact so that only 50% of the C\* can be recovered in carbon dioxide and iodoform. The observed recovery ( $33.6 + 16.5 = 50.1\%$ ) is in remarkably good agreement with the theoretical maximum.

As for the distribution of C\* it appears that considerably more is in the methyl than in the carboxyl group. Since a preferential reduction of carbon dioxide to a methyl group seems unlikely, the observed distribution suggests the existence of reversible exchange reactions between the carboxyl group and carbon dioxide. If such a reaction occurs, the radioactive

carbon in the carboxyl group will be diluted with carbon dioxide produced by the oxidation of uric acid. This point requires further investigation.

*Discussion.*—Although the above experiments show that carbon dioxide is reduced by *Cl. acidii-urici* to acetic acid in fermentations of purines, they do not prove that all the acetic acid is formed in this way. However, it is not improbable that this is the case. If acetic acid is derived entirely from carbon dioxide these "fermentations" would have to be regarded as *complete oxidations of purines with carbon dioxide acting as the ultimate oxidizing agent*. They would then be entirely analogous to the methane "fermentation" with acetic acid replacing methane as the reduction product. The generalized reaction would be



where  $\text{H}_2\text{A}$  represents the reducing agent, A its oxidation product. This interpretation is not without supporting evidence for it has been shown by Wieringa<sup>2</sup> that another *Clostridium* species is capable of oxidizing molecular hydrogen by means of carbon dioxide, the latter being reduced to acetic acid. In this connection it will be of interest to study the reduction of radioactive carbon dioxide by other acetic acid producing anaerobes.

In conclusion, it may be pointed out that these experiments furnish another example of the already large group of non-photosynthetic, heterotrophic organisms<sup>6</sup> that are able to reduce carbon dioxide. They also illustrate again the use of bacteria for the preparation of organic compounds containing radioactive carbon.<sup>3, 4</sup>

We are indebted to Dr. M. D. Kamen and Professor E. O. Lawrence for the radioactive carbon.

\* An extensive study of this organism will be published in the near future.

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## A COMPARISON OF THE METABOLISM OF IODINE AND OF ELEMENT 85 (EKA-IODINE)\*

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One of the procedures employed for the identification of the recently discovered halogen, eka-iodine (element 85), demonstrated that this new element is accumulated in the thyroid gland in a manner similar to iodine.<sup>1, 2, 3</sup> The following report includes a description of the investigation of the comparative metabolism of iodine and element 85 in guinea pigs.

For many years attempts had been made to discover the missing member of the halogen group. All efforts to isolate element 85 were unsuccessful although extremely sensitive tests were available by which quantities of the order of  $10^{-10}$  grams could be detected. Perrier and Segre in 1937<sup>4</sup> prepared and identified element 43 by the transmutation of molybdenum with the aid of the 37-inch Berkeley cyclotron. The amounts of element 43 obtained by this method were too minute to permit an investigation of its macroscopic chemical and physical properties. However, this artificially prepared element is radioactive and this characteristic made it possible to observe indirectly its chemical and physical properties and thereby to prove its identity. Pool and Quill<sup>5</sup> in 1938 stated that they had produced element 61 by the transmutation of neodymium in their cyclotron. These discoveries suggested that element 85 might be prepared in a similar manner. However, Oppenheimer<sup>6</sup> pointed out that to do so would require the transmutation of bismuth by alpha-particles (helium ions) possessing an energy in excess of 20,000,000 electron-volts. For this reason any attempts to produce element 85 had to await the availability of more energetic alpha-particles than could be obtained at the time when elements 43 and 61 were discovered. The recent completion of the 60-inch Berkeley cyclotron, which was designed and constructed by Lawrence and his associates, has made it possible to accelerate alpha-particles to energies exceeding 32,000,000 electron-volts. These extremely energetic nuclear particles were first successfully employed by Corson, MacKenzie and Segre<sup>1</sup> in the preparation of element 85. This new element is radioactive and has a half-life of 7.5 hours, but differs from all the other artificially produced radio-elements in that it emits alpha-particles.

*Preparation of the Radio-Halogens.*—Element 85 was produced by the bombardment of a water-cooled metallic bismuth target with 32,000,000 electron-volt alpha-particles. Approximately 200 mgm. of the irradiated bismuth were scraped from the surface of the target and placed in a small

molybdenum boat which was heated to 400 degrees Centigrade in an evacuated bell jar. The volatilized element 85 was collected upon a cold piece of glass suspended above the molten bismuth, dissolved in carbon tetrachloride and finally extracted with 10 cc. of 0.016 *N* sodium thiosulphate containing 4 mgm. of sodium carbonate.

Radio-iodine ( $I^{131}$ ) was prepared by the bombardment of metallic tellurium with 16,000,000 electron-volt deuterons. Following the deuteron bombardment in the cyclotron, the tellurium was removed from the target and transferred to a distilling flask equipped with a long delivery tube. Thirty cubic centimeters of 6 *N* nitric acid were then added to the flask and the mixture was heated in order to distill the liberated radio-iodine into a receiver containing 60 cc. of carbon tetrachloride. After the tellurium had been completely dissolved, the carbon tetrachloride was washed twice to remove the nitric acid which had been distilled over with the radio-iodine. The radio-iodine was removed from the carbon tetrachloride by extraction with 10 cc. of 0.016 *N* sodium thiosulphate. These two radio-halogens were separated from the bismuth and tellurium without the use of a carrier. The approximate quantities prepared for each animal were:  $5 \times 10^{-12}$  grams of radio-iodine (1 microcurie)\*\* and  $10^{-13}$  grams of element 85 (0.2 microcurie).

*Method of Study.*—The animals used in these experiments were guinea pigs of both sexes from 4 to 5 weeks of age whose weights ranged from 170 to 240 grams and which had been raised under the same conditions. In order to facilitate the collection of excreta, the animals were kept in metabolism cages for the duration of the experiments. Two-thirds of the animals received daily injections of thyrotropic hormone for a week before the radio-halogens were administered. The thyrotropic hormone produced symptoms of thyrotoxicosis and marked hyperplasia of the thyroid gland.

The uptake into the thyroid glands and the rates of excretion of radio-iodine and element 85 were observed in groups of thyrotoxic animals and normal controls. Equal and known quantities of radio-iodine and element 85, which had previously been made isotonic by the addition of sodium chloride, were administered together by subcutaneous injection to six thyrotoxic animals and four normal controls. Four hours later three of the thyrotoxic animals and two of the normal controls were sacrificed. Aliquot fractions of the urine and aqueous extracts of the feces collected during the interval together with the thyroids and samples of muscle, blood, liver and lymph nodes, were obtained for measurement of their content of radio-iodine and of element 85. The remaining three thyrotoxic animals and two normal controls were sacrificed 18 hours after injection, and excreta and samples of tissue were obtained as before. The study was repeated without the measurement of urine and feces in nine thyrotoxic animals and six normal controls. These were sacrificed in groups of three thyrotoxic

animals and two normal controls at the end of four, eighteen and sixty-five hours after administration of radio-iodine and element 85. The results of these experiments are shown in tables 1 and 2 and in figure 1.

The tissues and aliquot portions of the excreta were placed in flat ashing crucibles 4 cm. in diameter. One cubic centimeter of 0.1 *N* sodium hydroxide was added to each sample and the tissues were thoroughly macerated. The samples were then placed on a hot plate and evaporated to dryness at a temperature of 100 degrees Centigrade. This procedure made it possible to secure an even distribution of the material inside the crucibles. Aliquot fractions of the administered solutions of the two radio-halogens were measured out into these dishes together with 1 cc. of 0.1 *N* sodium hydroxide, and the mixture was evaporated to dryness. No corrections

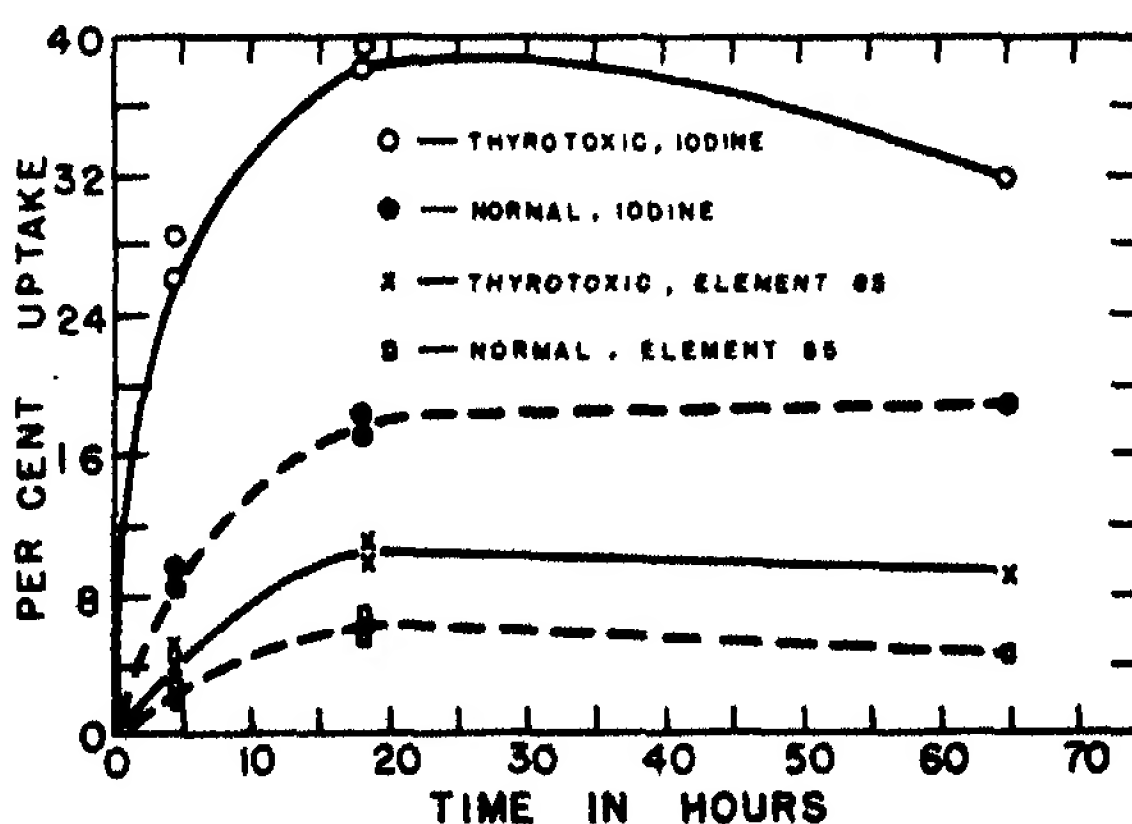


FIGURE 1

The uptake of radio-iodine and element 85 in the thyroid glands of normal and thyrotoxic guinea pigs.

were made for the self-absorption by the tissues and excreta of the radiation emitted by the radio-iodine and element 85 since the dry weight of the samples was less than 2.5 mgm. cm.<sup>-2</sup>. Corrections for the decay of radio-iodine and element 85 in the samples were not made since the standards and samples were measured at the same time.

The content of element 85 in the samples was determined by the use of a shallow ionization chamber which was connected to a linear amplifier and a mechanical recorder. This apparatus made it possible to measure quantitatively the number of alpha-particles emitted by the element 85 in the samples which also contained relatively large quantities of radio-iodine. Since the latter emits beta-particles, its radioactivity did not affect this type of ionization chamber. The radio-iodine in the samples was measured with a DuBridge type of vacuum tube electrometer several



days later in order to allow the element 85 to decay to a negligible quantity. This technique, therefore, made it possible to determine the amount of element 85 and radio-iodine in each sample.

*Results.*—The results recorded in tables 1 and 2 show that element 85 is accumulated in the thyroid gland and excreted in a manner similar to iodine. The curves in figure 1 show that the variations of uptake of radio-iodine and of element 85 by the thyroid glands of the different groups of animals were similar in these experiments although the uptake of the latter was consistently less. The rates of excretion of radio-iodine and element 85 were almost identical and the kidneys apparently acted as the main channel of elimination. The proportion of the two elements excreted in the feces was probably due to contamination from the urine. The contents of the radio-iodine and of element 85 in the other tissues examined was found to be less than 1 per cent of their concentration in the thyroid gland.

TABLE 1

UPTAKE BY THE THYROID GLANDS AND URINARY AND FECAL EXCRETIONS OF RADIO-IODINE AND ELEMENT 85 IN NORMAL AND THYROTOXIC GUINEA PIGS

NUMBER	ANIMALS TYPE	HOURS AFTER ADMINIS- TRATION	UPTAKE BY THYROID		URINARY EXCRETION		FECAL EXCRETION	
			IODINE <sup>131</sup>	ELEMENT 85	IODINE <sup>131</sup>	ELEMENT 85	IODINE <sup>131</sup>	ELEMENT 85
3	Thyrototoxic	4	26.1%	4.2%	13.0%	16.0%	1.0%	0.7%
2	Normal	4	8.5%	3.4%	12.4%	8.8%	0.8%	0.4%
3	Thyrototoxic	18	38.3%	10.7%	25.7%	34.0%	5.7%	8.7%
2	Normal	18	16.9%	5.4%	37.2%	36.0%	17.0%	13.0%

*Discussion.*—The thyroid gland is unique in that it possesses the ability to accumulate iodine selectively in relatively large quantities. This property is particularly striking because the iodine content of the blood averages less than one part in ten million while the thyroid gland normally contains approximately one part in a thousand. This indicates that the thyroid gland can concentrate the iodine it receives from the blood by a factor of ten thousand. Many organs of the body are capable of storing certain elements, such as phosphorus, magnesium and calcium in the bones, iron in the blood and the liver, and zinc in the pancreas. However, no organ in the body has the power of concentration and storage possessed by the thyroid gland.

A relationship between the periodicity of the chemical and the physical properties of the elements and their physiological action was discovered by James Blake and fully discussed by him in 1848.<sup>7</sup> He studied the effects of nearly all the elements known at that time on the circulation, respiration and the central nervous system of dogs. He was thus able to arrange the elements in groups on the basis of similarity of physiological action. Blake's observations have been confirmed by many investigators both for



the elements available at the time of his experimental studies and for those which have been discovered subsequently.

Since element 85 is a halogen and iodine is its closest homologue, it was felt that further proof of its identity could be secured by a comparison of the biological properties of this new element with iodine. The results of the experiments described in this report show that element 85 shares with iodine the unique property of being selectively accumulated and stored in the thyroid gland. Further proof of the halogen character of this new element is demonstrated by the rapidity of its excretion while all the other heavy elements, such as radium, thorium, lead, bismuth and thallium, tend to be excreted slowly.

The fact that element 85 is retained in the thyroid suggests that it is probably held in firm chemical combination. Many years ago Marine<sup>8</sup> presented clinical data suggesting that once iodine is stored in the thyroid gland its release is very slow. We have recently confirmed his views by directly measuring the rates of uptake and storage of radio-iodine in the

TABLE 2  
UPTAKE OF RADIO-IODINE AND ELEMENT 85 BY THE THYROID GLANDS OF NORMAL AND THYROTOXIC GUINEA PIGS

NUMBER	ANIMALS TYPE	HOURS AFTER ADMINISTRATION	UPTAKE BY THYROID	
			IODINE <sup>131</sup>	ELEMENT 85
3	Thyrototoxic	4	28.3%	4.3%
2	Normal	4	9.3%	2.9%
3	Thyrototoxic	18	39.8%	9.7%
2	Normal	18	18.5%	7.1%
3	Thyrototoxic	65	31.8%	8.8%
2	Normal	65	18.8%	4.5%

intact thyroid glands of normal human subjects and of patients suffering from various types of thyroid disease.<sup>9, 10, 11</sup> The relatively short half-life of element 85 makes it difficult to follow its metabolism in the thyroid for more than three days. However, the fact that no significant loss from the thyroid glands of either radio-iodine or element 85 was observed during this interval indicates that element 85 is held in thyroid tissue as firmly as iodine.

The increased accumulation of element 85 in the hyperplastic thyroid glands of guinea pigs suggests that this new element may be of potential value in the treatment of human thyroid disorders in which the functional activity of the thyroid gland is abnormally increased. An investigation of the relation between the microscopic anatomy of thyroid tissue and the deposition of radio-iodine has shown that this element is concentrated in the most actively functioning portions of the thyroid glands of goiterous patients.<sup>12</sup> If element 85 is found to behave in a manner similar to radio-iodine in the thyroid of patients with hyperthyroidism, then it should be

superior to radio-iodine as a possible therapeutic agent. This is due to the fact that element 85 emits alpha-particles which have an average energy of 4,000,000 electron-volts, while the beta-rays from the radio-iodine ( $I^{131}$ ) only have an average energy of approximately 200,000 electron-volts. Each alpha-particle gives up its energy in a material such as thyroid tissue within a distance of less than 50 microns, while the beta-rays from radio-iodine lost most of their energy in a distance of 500 microns. The density of ionization produced by these alpha-particles is over two hundred-fold greater than that resulting from the radio-iodine beta-rays. This means that, since the range of the alpha-particles is so short, the thyroid cells which accumulate element 85 in the largest quantities would suffer the brunt of its radiation. The action of radio-iodine in the thyroid gland would be much more diffuse because of the longer range of the beta-rays. Therefore there would be a considerable radiation effect upon the thyroid cells lying at a distance from those areas in which relatively large quantities of radio-iodine have been concentrated.

*Summary.*—A comparison of the metabolism of element 85 and that of iodine has demonstrated that these two halogens are stored in thyroid tissue and are excreted in a similar manner.

*Acknowledgments.*—The authors wish to express their appreciation to Professor Ernest O. Lawrence and his co-workers in the Radiation Laboratory for the preparation of radio-iodine and element 85. The physical measurements were made with the assistance of Drs. Dale R. Corson and Kenneth R. MacKenzie of the Radiation Laboratory. The criticisms and suggestions by Dr. John H. Lawrence of the Division of Medicine, Professor Edwin McMillan and Dr. Emilio Segre of the Radiation Laboratory and Professor Chauncey D. Leake of the Division of Pharmacology are gratefully acknowledged. The experimental animals were supplied by Dr. Herbert M. Evans and his staff of the Institute of Experimental Biology and the thyrotoxic animals were prepared by the administration of a highly purified and potent thyrotropic hormone produced in their laboratory.

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\*\* 1 microcurie is defined here as the quantity of radioactive material in which  $3.7 \times 10^4$  atoms disintegrate per second.

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## SURFACES OF MINIMAL CAPACITY

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It is asked whether, given a closed curve  $s$  in space, there exists among the surfaces of which  $s$  is the complete boundary, one for which the capacity is a minimum. This problem is investigated for the case of a closed curve which is itself of zero capacity and such that there exists a one-one continuous transformation  $\xi$  which carries a large sphere, containing  $s$  in its interior, into itself in such a way that  $s$  is equivalent to a circle  $\sigma$ , and the points of the surface of the sphere remain invariant. We may regard  $\xi$  as extended in the rest of space to be the identical transformation.

If  $S$  is a surface bounded by  $s$ , which except in the neighborhood of  $s$  is composed of a finite number of sufficiently smooth pieces, and  $V(M)$  is the conductor potential for  $S$  it may be shown that a necessary condition for the capacity to be a minimum is the equality

$$\frac{dV(Q)}{dn+} = \frac{dV(Q)}{dn-}, \quad (1)$$

holding for all points  $Q$  on the smooth pieces which constitute  $S$ ,  $n+$  and  $n-$  being oppositely directed normals at the point  $Q$ . A statement equivalent to (1) is the integral relation

$$\int_S \int \left( \frac{d}{dn_Q} \frac{1}{QP} \right) d\mu(e_P) = 0, \quad (2)$$

where  $\mu(e)$  is the distribution of positive mass on  $S$  which generates the conductor potential.

The principal theorem is the following:

**THEOREM.** *There exists a unique surface  $S$  bounded by  $s$  which satisfies the equation (1) at all points of its smooth pieces; and except for nodal lines and points, this surface is analytic.*

In fact, the condition (1) is the condition that  $V(M)$  may be extended analytically and as a harmonic function across  $S$  in the neighborhood of any smooth piece of it. It may be proved then that  $S$  is the whole of the level surface  $V = 1$  of this harmonic function.

The proof of the theorem is obtained by setting up this (multiple valued) harmonic function independently. It is necessary to apply the Schwarz alternating process to multiple leaved Riemann manifolds in three dimensions. First we consider the infinite 3-space as bounded internally by a torus about  $s$ —that is, the image by  $\xi$  of a torus about  $\sigma$ —and introduce the two leaved Riemann manifold into this space in order to obtain a two valued harmonic function which takes on the value 1 on the surface of the torus and with its two branches the values 0 and 2, respectively, at  $\infty$ .

By letting the torus shrink down to the curve  $s$  we obtain a function  $v(M)$  with the following properties:

( $\alpha$ ) Either branch of the function is harmonically extensible along any curve in space which does not meet  $s$ .

( $\beta$ ) The function is bounded and one of its two values tends to 0 at  $\infty$ , the other to 2.

( $\delta$ ) If the two values  $v_1(M)$ ,  $v_2(M)$  of  $v$  are distinct at  $M$ , a closed path which starts from  $M$  and loops  $s$  once or an odd number of times, returning to  $M$ , carries  $v_1(M)$  into  $v_2(M)$ , and vice versa, whereas if the closed path does not loop  $s$  it carries each value back into itself.

( $\lambda$ ) At every point  $M$  not on  $s$ ,  $v_1(M) + v_2(M) = 2$ .

( $\gamma$ ) Every point of  $s$  is a limit point of the level surface  $v_1(P) = v_2(P) = 1$ .

It may be shown by an extension of Kellogg's fundamental uniqueness theorem<sup>1</sup> that the properties ( $\alpha$ ), ( $\beta$ ), ( $\delta$ ) serve to determine the function  $v(M)$  uniquely; of these, ( $\alpha$ ) is required by equation (1), ( $\beta$ ) by the fact that  $V(M)$  is the conductor potential and ( $\delta$ ) is an interpretation of the requirement that  $S$  be bounded by  $s$ . Conversely, by these properties, including ( $\lambda$ ) and ( $\gamma$ ), it is seen that the function defined as the minorant of  $v_1(M)$ ,  $v_2(M)$  is the conductor potential of  $S$  and satisfies (1) at every point. The relation (2) shows that the surface  $S$  lies in the convex envelope of the curve  $s$ .

The problem under discussion is more intimately connected with three dimensions than that of Plateau. The difference in type between the two is in fact made clear by the uniqueness theorem based on ( $\alpha$ ), ( $\beta$ ) and ( $\delta$ ). For instance, if we commence with a plane curve which bounds a figure composed of two circles exterior to each other but connected by a strip, and deform the curve by bending the two circles towards each other, the Plateau figure persists beyond the situation in which a second solution is stable; but in the present problem a nodal point or line must develop, and the figure changes gradually into one in which a surface of the same connectivity as the original one appears as a strip joining the approximately

parallel circumferences of the two circles. Similarly a two-sided figure may be made to change gradually into a one-sided one.

In particular, if  $s$  is a plane curve the solution is evidently the portion of the plane enclosed by it. If  $s$  lies on a closed convex surface,  $S$  is essentially two-sided. The functions  $v(M)$ ,  $V(M)$  are in general not necessarily continuous on  $s$ , for some of the points of  $s$  may be irregular boundary points for the conductor potential  $V(M)$ .

In two dimensions a similar problem has been considered in particular cases as a generalization of a well-known theorem of Koebe on conformal mapping. Pólya and Szegő consider it as a problem in transfinite diameter for a two point boundary in the plane, where the solution is the segment joining them.<sup>2</sup> I am indebted to Professor Szegő for the citations<sup>3</sup> with respect to the problem in the plane. In particular Grötzsch demonstrates by methods of conformal mapping the uniqueness of the solution for an arbitrary finite number of points in the plane.

<sup>1</sup> O. D. Kellogg, "Foundations of Potential Theory," p. 335 (1920).

<sup>2</sup> G. Pólya and G. Szegő, "Transfiniter Durchmesser ebener und räumlicher Punktmengen," *Jour. für die reine und angewandte Mathematik*, **165**, 4-49 (1931).

<sup>3</sup> L. Bieberbach, "Ueber die Koeffizienten derjenigen Potenzreihen, welche eine schlichte Abbildung des Einheitskreises vermitteln," *Akademie der Wissenschaften, Berlin, Sitzungsberichte*, 940-955 (1916); G. Pólya, "Beitrag zur Verallgemeinerung des Verzerrungssatzes auf mehrfach zusammenhängende Gebiete," *Ibid.*, 228-232 and 280-282 (1928-2), 55-62 (1929-2); H. Grötzsch, "Ueber ein Variationsproblem der konformen Abbildung," *Akademie der Wissenschaften, Leipzig, Berichte*, **82**, 251-263 (1930).

## THE POLYNOMIAL OF MITTAG-LEFFLER

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1. The polynomial  $g_n(z) = 2zF(1 - n, 1 - z; 2; 2)$  occurs as a coefficient in the expansions

$$(1 + t)^s(1 - t)^{-s} = 1 + \sum_{n=0}^{\infty} g_n(z)t^n, \quad |t| < 1 \quad (1)$$

$$2ze^t F(1 - z; 2; -2t) = \sum_{n=1}^{\infty} g_n(z)t^{n-1}/(n-1)! \quad (2)$$

It was used by Mittag-Leffler<sup>1</sup> in a study of the analytical representation of the integrals and invariants of a linear homogeneous differential equa-

tion in which he made use of a conformal mapping of the  $t$ -plane on the  $w$ -plane by means of a relation

$$w = w_0(1 + t)^z(1 - t)^{-z} \quad (3)$$

in which the index  $z$  was an imaginary quantity  $2b/i\pi$ . The first expansion was used later in his researches on the analytical representation of a uniform branch of a monogenic function and was connected with some other expansions. The second expansion is new only in notation being merely a particular case of a well-known expansion in which the coefficients are hypergeometric functions.<sup>2</sup>

Pidduck<sup>3</sup> used an expansion equivalent to (1) in his researches on the propagation of a disturbance in a fluid acted upon by gravity. He gave the recurrence relation

$$g_n(z + 1) - g_{n-1}(z + 1) = g_n(z) + g_{n-1}(z) \quad (4)$$

which is an immediate consequence of the fact that the generating function  $G(z, t) = (1 + t)^z(1 - t)^{-z}$  satisfies the functional equation

$$(1 - t)G(z + 1, t) = (1 + t)G(z, t). \quad (5)$$

A second recurrence relation

$$ng_n(z) = (n - 2)g_{n-2}(z) + 2zg_{n-1}(z) \quad (6)$$

has been given by Belorizky;<sup>4</sup> it is a consequence of the fact that

$$(1 - t^2)dG/dt = 2zG. \quad (7)$$

When  $|u|$  is sufficiently small the relation

$$\sum_{n=0}^{\infty} u^n (1 + t)^n (1 - t)^{-n} = 1 + 2u \sum_{m=1}^{\infty} t^m (1 + u)^{m-1} (1 - u)^{-m-1} \quad (8)$$

shows that if  $n > 0$

$$2u(1 + u)^{n-1} (1 - u)^{-n-1} = \sum_{m=0}^{\infty} u^m g_n(m) \quad (9)$$

$$\begin{aligned} \text{and so } 2g_m(n + 1) &= g_n(m - 1) + 2g_n(m) + g_n(m + 1) \\ &= g_{n+1}(m + 1) - g_{n+1}(m - 1). \end{aligned}$$

Consequently, if  $n \geq 1$

$$2g_m(n) = g_n(m + 1) - g_n(m - 1). \quad (10)$$

2. The polynomial  $g_n(z)$  may be generalized by writing

$$(1 + t)^{z+r} (1 - t)^{-z} = \sum_{n=0}^{\infty} t^n g_n(z, r). \quad (11)$$



If  $(1 + t)^r$  is expanded by the binomial theorem

$$(1 + t)^r = \sum_{n=0}^{\infty} (r/, n) t^n \quad (12)$$

it is found that

$$g_n(z, r) = \sum_{s=0}^n (r/, s) g_{n-s}(z), \quad (13)$$

the series terminating earlier when  $r$  is a positive integer  $< n$ .

The expansion (11) is a particular case of the more general expansion of W. Gordon<sup>5</sup> and J. L. Lagrange<sup>6</sup>

$$(1 + t)^{b-c} [1 + (1 - z)t]^{-b} = \sum_{n=0}^{\infty} t^n (-c/, n) F(-n, b; c; z) \quad |t| < 1, |t - tz| < 1. \quad (14)$$

It follows from this expansion that when  $r = 0, 1, \dots, n - 1$

$$g_n(z, r) = (r/, n) F(-n, z; -r; 2). \quad (15)$$

Pidduck considered the case  $r = -1$  and so his second coefficient is with a different notation

$$g_n(z + 1, -1) = (-)^n F(-n, z + 1; 1; 2) = F(-n, -z; 1; 2). \quad (16)$$

A second expression for  $g_n(z, r)$  in terms of the hypergeometric series is a consequence of Euler's relation

$$F(a, b; c; x) = (1 - x)^{-a} F\left(a, c - b; c; \frac{x}{x - 1}\right) \quad (17)$$

which gives the formula

$$g_n(z, r) = (-)^n (r/, n) F(-n, -z - r; -r; 2). \quad (18)$$

3. The relation

$$F(-n, -z - r; -r; t) = [(z + r/, n)/(r/, n)] (-t)^n F(r - n + 1, -n; z + r - n + 1; t^{-1}) \quad (19)$$

indicates also that

$$g_n(z, r) = (z + r/, n) 2^n F(r - n + 1, -n; z + r - n + 1; 1/2) \quad (20)$$

and Euler's relation

$$F(a, b; c; x) = (1 - x)^{c-a-b} F(c - a, c - b; c; x) \quad (21)$$

indicates that

$$g_n(z, r) = (z + r/, n) 2^{-z} F(z, r + z + 1; r + z - n + 1; 1/2). \quad (22)$$

This formula gives an estimate of  $g_n(z, r)$  for large positive values of  $n$  when  $r + z$  is not one of the numbers  $0, 1, 2, \dots, n-1$  and is also independent of  $n$ . The hypergeometric series actually gives a convergent expansion in a series of inverse factorials when  $g_n(z, r)$  is regarded as a function of  $n$ . Similarly

$$F(-n, b; c; x) = x^n [(b, n)/(c, n)] (-)^n F(1 - n - c, -n; 1 - n - b; z) \quad (23)$$

where  $z = 1/x$  and (21) gives for  $|x| > 1$

$$F(-n, b; c; x) = x^{b-c} (x-1)^{n+c-b} (b, n)/(c, n) (-)^n F(c-b, 1-b; 1-n-b; z). \quad (24)$$

Hence when  $|x| > 1$  and  $n$  is large

$$F(-n, b; c; x) \sim (-)^n x^{b-c} (x-1)^{n+c-b} (b, n)/(c, n). \quad (25)$$

Negative integral values of  $c$  which made  $(c, n) = 0$  should be excepted. Also negative integral values of  $b-1$  should be excepted unless  $b=c$ .

4. Definite integrals for  $g_n(z)$  and  $g_n(z, r)$  may be derived from the well-known definite integrals for the hypergeometric function. In particular, if  $-1 < z < 1$ ,  $n > 0$

$$B(z, 1-z)g_n(z) = \int_{-1}^1 t^{n-1} (1+t)^z (1-t)^{-z} dt. \quad (26)$$

This result may be written in the alternative form

$$g_n(z) = (1/\pi) \sin(\pi z) \int_{-\infty}^{\infty} e^{uz} (\tanh^{1/2} u)^n du / \operatorname{sh} u \quad (27)$$

Differentiating  $m$  times with respect to  $z$  we find that

$$g_n^{(m)}(z) = \left( \frac{1}{2\pi i} \right) \int_{-\infty}^{\infty} [e^{z(u+i\pi)} (u+i\pi)^m - e^{z(u-i\pi)} (u-i\pi)^m] (\tanh^{1/2} u)^n du / \operatorname{sh} u \quad (28)$$

Putting  $z = 0$  we obtain the formula

$$g_n^{(m)}(0) = \left( \frac{1}{2\pi i} \right) \int_{-\infty}^{\infty} [(u+i\pi)^m - (u-i\pi)^m] (\tanh^{1/2} u)^n du / \operatorname{sh} u \quad (29)$$

for the coefficient of  $t^n$  in Mittag-Leffler's expansion

$$[2Q_0(t)]^m = \left[ \log \frac{1+t}{1-t} \right]^m = \sum_{n=-\infty}^{\infty} t^n g_n^{(m)}(0). \quad (30)$$

When  $|R(z)| < 1$  there is a formula

$$g_n(z) = (1/\pi) \int_0^\pi (\cot^{1/2} u)^z \cos(1/2 \pi z - nu) du \quad (31)$$

which may be established with the aid of the recurrence relation and may be regarded as holding for negative integral values of  $n$  as well as for positive integral values. Since the recurrence relation gives  $g_n(z) = 0$  when  $n$  is a negative integer we have for all positive integral values of  $n$

$$g_n(z) = (2/\pi) \int_0^\pi (\cot \frac{1}{2}u)^n \cos(\frac{1}{2}\pi z) \cos(nu) du \quad (32)$$

$$g_n(z) = (2/\pi) \int_0^\pi (\cot \frac{1}{2}u)^n \sin(\frac{1}{2}\pi z) \sin(nu) du \quad (33)$$

If  $n > 0$  and  $|R(z)| < 1$  the formula

$$g_n(z) = (1/\pi) \int_0^{2\pi} (1 + e^{ia})^n (2 + e^{ia})^{n-1} e^{-ina} da \quad (34)$$

may be derived from the series for  $g_n(z)$  in terms of binomial coefficients. A corresponding formula may be obtained for  $g_n(z, r)$ . Another type of formula for  $g_n(z)$  is obtained by starting from the expansion of Liouville<sup>7</sup> and Lerch<sup>8</sup>

$$\exp\left(x \frac{t-1}{t+1}\right) = \sum_{n=0}^{\infty} k_{2n}(x) t^n \quad (35)$$

in which  $k_{2n}(x) = e^{-x} (-)^{n-1} (2x) F(1-n; 2; 2x)$  for  $n > 1$   
 $k_0(x) = e^{-x}$

The formula in question is

$$\Gamma(z) g_n(z) = \int_0^\infty x^{z-1} k_{2n}(x) dx, \quad R(z) > -1 \text{ for } n > 0 \quad (36)$$

A corresponding formula for  $g_n(z, r)$  is

$$\Gamma(z) g_n(z, -m-1) = (-)^n \int_0^\infty e^{-x} L_n^m(2x) x^{z-1} dx, \quad R(z) > 0 \quad (37)$$

where  $L_n^m(u)$  is the generalized polynomial of Laguerre. With the notation of Sonine's polynomial

$$g_n(z, -m-1) \Gamma(z) = \Gamma(m+n+1) \int_0^\infty e^{-x} T_m^n(2x) x^{z-1} dx. \quad (38)$$

Another expression for  $g_n(z)$  is obtained from the expansion

$$(1-u)^{-2} \exp\left[-x \left(\frac{1+u}{1-u}\right)^2\right] = \sum_{n=0}^{\infty} u^n T_n^*(x)$$

which indicates that

$$\int_0^\infty T_n^*(x)x^{z-1}dx = \frac{\Gamma(z)}{2-4z} (n+1)g_{n+1}(1-2z), R(z) > 0 \quad (39)$$

TABLE OF  $g_n(m)$

$n$	0	1	2	3	4	5	6	7	8	9	10
$m$											
0	1	0	0	0	0	0	0	0	0	0	0
1	1	2	2	2	2	2	2	2	2	2	2
2	1	4	8	12	16	20	24	28	32	36	40
3	1	6	18	38	66	102	146	198	258	326	402
4	1	8	32	88	192	360	608	952	1408	1992	2720
5	1	10	50	170	450	1002	1970	3530	5890	9290	14002
6	1	12	72	292	912	2364	5336	10836	20256	35436	58728
7	1	14	98	462	1666	4942	12642	28814	59906	115598	209762
8	1	16	128	688	2816	9424	27008	68464	157184	332688	658048
9	1	18	162	978	4482	16722	53154	148626	374274	864146	1854882
10	1	20	200	1340	6800	28004	97880	299660	822560	2060980	4780008

$g_0(z) = 1, g_1(z) = 2z, 3g_3(z) = 4z^3 + 2z, 3g_4(z) = 2z^4 + 4z^2, g_2(z) = 2z^2,$   
 $15g_5(z) = 4z^5 + 20z^3 + 6z, 45g_6(z) = 4z^6 + 40z^4 + 46z^2.$

$$g_n(-z) = (-)^ng_n(z)$$

$$g_{2n}(1/2) = g_{2n+1}(1/2) = \frac{1.3.5 \dots (2n-1)}{2.4.6 \dots 2n} = (n-1/2/n)$$

<sup>1</sup> Mittag-Leffler, G., *Acta Math.*, **15**, 1-32 (1891); **24**, 183-245 (1901).  
<sup>2</sup> Appell, P., *Ann. école norm.*, (2) **9**, 119-144 (1880).  
<sup>3</sup> Pidduck, F. B., *Proc. Roy. Soc. (London)*, **A83**, 347-356 (1910); **86**, 396-405 (1912).  
<sup>4</sup> Belorizky, D., *Comptes Rendus*, **195**, 1222-1224 (1932).  
<sup>5</sup> Gordon, W., *Ann. der Physik*, (5) **2**, 1031-1056 (1929).  
<sup>6</sup> Lagrange, J. L., *Oeuvres*, **2**, 173-234 (1770-1773), see p. 220.  
<sup>7</sup> Liouville, J., *J. de Math.*, (2) **2**, 433-440 (1857).  
<sup>8</sup> Lerch, M., *J. f. Math.*, **130**, 47-65 (1905).

## BINARY FORMS

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Elsewhere<sup>1</sup> the author has proved that, for a field  $K$  with  $(n + 1)$  or more elements, a form  $F$  of degree  $n$  with a symmetric matrix of coefficients can be written for a finite  $\sigma$  as a linear combination of  $\sigma$   $n$ th powers of linear forms with coefficients in  $K$ . The existence of such "representations" of  $F$  having been established it remains to determine a method of constructing all such representations. In the present note this problem of construction is solved for binary forms by the introduction of certain forms termed "associates." From these results solutions can be obtained for such classical problems as the equivalence and reducibility of forms. The restriction of the treatment to binary forms is not a serious one since most of the theory can be generalized to forms in any number of variables.

1. *Definitions.*—Let  $F$  be a binary form of degree  $n$  with coefficients in a field  $K$  for which the *characteristic* is greater than  $n$ . We can write

$$F = \sum_{i=1}^{\sigma} \lambda_i L_i^n, \quad (1.1)$$

where the  $\lambda$ 's are elements of  $K$ , and the  $L$ 's are linear forms with coefficients in  $K$ . The sum (1.1) is termed a  $\sigma$ -*representation* of  $F$  with respect to  $K$ . If  $\sigma$  takes on its smallest value in (1.1) for  $K$ , the integer  $\sigma$  is termed the *minimal number* of  $F$  for  $K$ , and the associated *representation* is called *minimal*. The restriction above on the characteristic of  $K$  is made throughout this paper so that  $F$  will have a unique symmetric matrix of coefficients. Unless the field is specifically mentioned the theorems of this paper are understood to be *valid for fields with this restriction*. We write  $F$  as

$$\sum_1^2 a_{ij \dots k} x_i x_j \dots x_k,$$

where the indices range over 1, 2, and the matrix  $A = (a_{ij \dots rs \dots k})$  is completely symmetric. We arrange the elements of  $A$  in a 2-way display  $\|a_{\alpha\beta}\|$  where  $\alpha$  is the set of indices  $i, j, \dots, r$  and  $\beta$  the set of indices  $s, \dots, k$  of  $A$ . In the matrix  $\|a_{\alpha\beta}\|$  the indices  $i, j, \dots, r$  vary with the rows of  $\|a_{\alpha\beta}\|$  whereas the remaining indices vary with the columns of  $\|a_{\alpha\beta}\|$ . The rank of  $\|a_{\alpha\beta}\|$  is a 2-way rank of  $F$  studied elsewhere.<sup>1</sup> We write  $F$  in the standard form

$$a_0 x^n + n a_1 x^{n-1} y + \frac{n(n-1)}{2!} a_2 x^{n-2} y^2 + \dots + a_n y^n.$$

We shall make extensive use of the following matrix:

$$A_{\sigma} = \begin{vmatrix} a_0 & a_1 & a_2 & a_3 & \dots & a_{\sigma} \\ a_1 & a_2 & a_3 & a_4 & \dots & a_{\sigma+1} \\ & & \cdot & & & \cdot \\ & & & \cdot & & \\ a_{n-\sigma} & a_{n-\sigma+1} & a_{n-\sigma+2} & a_{n-\sigma+3} & \dots & a_n \end{vmatrix}.$$

Let  $\sigma$  be the number of indices in the set  $s, \dots, k$ . The rank of  $A_{\sigma}$  equals the rank of  $\|a_{\alpha\beta}\|$ . We shall term the rank of  $A_{\sigma}$  the  $\sigma$ -rank of  $F$ . We shall permit  $\sigma$  to take on the value  $n$ . Let  $r$  and  $\xi$  denote the vectors

$$\|y^{\sigma} \quad -y^{\sigma-1}x \quad y^{\sigma-2}x^2 \quad \dots \quad \neq x^{\sigma}\|, \quad \begin{vmatrix} \xi_0 \\ \cdot \\ \cdot \\ \cdot \\ \xi_{\sigma} \end{vmatrix}$$

respectively. If the equation

$$\|A_{\sigma}\|.\xi = 0 \tag{1.2}$$

has a solution for  $\xi \neq 0$ , the equation defines a form  $r\xi$  in  $x$  and  $y$  up to a constant factor, which form we shall term the  $\sigma$ -associate of  $F$ . In the case where the 2-rank of a binary cubic is 2, the 2-associate can be taken to be the Hessian of  $F$ .

2. *Relation between Representations, and the Determination of the Minimal Number.*—By equating coefficients in (1.1) and using various theorems on vectors, we can prove the basic theorem of the present note, which theorem follows.

**THEOREM 2.1.** *A binary form  $F$  of degree  $n$  has a representation (1.1) with  $\sigma \leq n$  and  $s$  of the  $L$ 's linearly independent in pairs if and only if the  $s$ -associate of  $F$  can be factored into a product of these  $L$ 's.*

If a  $\sigma$ -rank of  $F$  is  $\sigma + 1$  there does not exist a  $\sigma$ -associate  $F_{\sigma}$  of  $F$  in the sense that  $F_{\sigma} \neq 0$ . From this and Theorem 2.1 we obtain the following result.

**THEOREM 2.2.** *The minimal number of a binary form  $F$  is the smallest value of  $\sigma$  such that the  $\sigma$ -rank of  $F$  is not greater than  $\sigma$ , and the  $\sigma$ -associate of  $F$  can be factored for some choice of the coefficients of  $F_{\sigma}$  into linear factors, linearly independent in pairs.*

For algebraically closed fields whether or not a binary form has a repeated factor can be recognized by the use of resultants, whence a computation of ranks and resultants yields the minimal number for these fields.

3. *Associates.*—That the associates are intimately related to each other is brought out in the following theorem.

**THEOREM 3.1.** *If  $\rho \geq \sigma$ , and  $\sigma$  is such that the  $\sigma$ -associate  $F_{\sigma}$  of a binary*



form  $F$  exists, the  $\rho$ -associate  $F_\rho$  of  $F$  can be factored into  $F_\rho G$  where  $G$  is arbitrary.

The associates of a form  $F$  transform covariantly with  $F$ .

4. *Ranks.*—A study of the matrices of the type  $A_\sigma$  of §1 yields the following theorem.

**THEOREM 4.1.** *For a binary form  $F$  of degree  $n$  there exists a number  $\gamma$  such that the  $(n - \sigma)$  and  $\sigma$ -ranks of  $F$  equal  $(\sigma + 1)$  for  $\sigma = 0, 1, 2, \dots, (\gamma - 1)$  while the remaining 2-way ranks of  $F$  equal  $\gamma$ .*

The smallest value of  $\sigma$  for which the  $\sigma$ -associate of  $F$  exists is  $\gamma$ .

Like the minimal number, the  $\sigma$ -ranks of  $F + \lambda L^n$  differ from the corresponding ranks of  $F$  by at most 1. These results have numerous applications to be given elsewhere.

5. *Uniqueness.*—By use of the associates one can show that a  $\sigma$ -representation of a binary form  $F$  is minimal if  $\sigma \leq (n/2) + 1$ . This representation is unique in the usual sense if  $\sigma \leq (n + 1)/2$ . For the complex field the minimal number of the "general" binary form  $F$  of odd degree is  $(n + 1)/2$ , whence the minimal representation of  $F$  is unique. We use "general" here in the sense of Sylvester<sup>2</sup> and Elliott.<sup>3</sup> The present results shed considerable light on the theory of binary forms developed by these men.

If the minimal representation of a form  $F$  is unique, the question of its equivalence to another form has a simple answer.

6. *Minimal Number.*—From relations between the minimal number and various factorization properties of  $F$  for the complex field it follows that the minimal number of a binary form  $F$  attains the value  $n$  if and only if  $F = L^{n-1}M$  for linear forms  $L$  and  $M$ . That the minimal number does not exceed  $n$  was proved elsewhere.<sup>4</sup>

<sup>1</sup> Oldenburger, *Proc. Nat. Acad. Sci.*, **24**, 193–198 (1938).

<sup>2</sup> Sylvester, *Phil. Mag.*, **94**, 391–410 (1851).

<sup>3</sup> Elliott, *Algebra of Quantics*.

<sup>4</sup> Oldenburger and Porges, *Bull. Amer. Math. Soc.*, **5** (1940) (in press).

## SUBGROUPS OF THE GROUPS WHOSE ORDERS ARE BELOW THIRTY

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A subgroup which is neither the identity nor the entire group  $G$  is commonly called a proper subgroup of  $G$ . In the present article only proper subgroups will be implied by the term subgroup unless the contrary is explicitly stated. The term total number of subgroups will be used whenever the identity and  $G$  itself are included among the subgroups of  $G$  so that the total number of subgroups of  $G$  is always equal to the number of the proper subgroups of  $G$  increased by 2 unless  $G$  is the identity. There are 88 groups whose orders are below 30 and these furnish many useful illustrations of group properties which can easily be verified on account of the low orders of the groups. They therefore provide a useful field for study in view of the fact that the larger groups which are not of prime orders can usually be most conveniently studied by means of their subgroups and have been thus studied since the time of J. L. Lagrange (1736–1813).

Among the general theorems, relating to the number of the subgroups of  $G$  the following are especially useful. The cyclic group of order  $p^m$ ,  $p$  being any prime number, contains exactly  $m - 1$  subgroups and hence there is at least one group which contains an arbitrary given number of subgroups. In the particular cases when this given number is either 5 or 11 it has recently been proved that there is no other group which contains exactly this number of subgroups. The abelian group of order  $p^m$  and of the type  $m - 1, 1$  contains exactly  $p + 1$  subgroups of order  $p, p^2, \dots, p^{m-1}$ . This is also the case with respect to the non-abelian group which is conformal therewith and exists in all cases when  $m > 2$ , except when  $p = 2$  and  $m = 3$ . There exists a non-abelian group of order  $pq$ ,  $p$  and  $q$  being distinct prime numbers and  $p > q$ , if and only if  $p - 1$  is divisible by  $q$ , and the number of its subgroups is then  $p + 1$ . When  $p - 1$  is not divisible by  $q$  the group of order  $pq$  contains exactly 2 subgroups and is cyclic.

Suppose that a given group  $G$  has exactly  $k$  subgroups. It is then possible to construct in the following manner an arbitrary number of groups which separately contain exactly  $2k + 2$  subgroups. To do this it is only necessary to find this arbitrary number of prime numbers which are separately prime to the order of  $G$  and then to form successively the direct products of  $G$  and the separate groups whose orders are equal to these separate prime numbers. For instance, the symmetric group of order 6 contains 4 subgroups and the prime numbers 5, 7, 11, etc., are separately prime to the order of this symmetric group. Hence each of the direct products of

this symmetric group and the group of one of the orders 5, 7, 11, etc., contains exactly 10 subgroups. The resulting groups may clearly be used similarly to construct other infinite systems of groups containing separately exactly  $4k + 4 + 2$  subgroups, etc. The entire system thus obtained is composed of abelian or of non-abelian groups as  $G$  is either abelian or non-abelian.

The total number of the subgroups of an abelian group whose order is not a power of a prime number is the product of the total numbers of the subgroups in its Sylow subgroups. In particular, the total number of such subgroups cannot be a prime number, and the determination of the total number of the subgroups of any abelian group is reduced to the determination of the subgroups of prime power abelian groups. In the special case when the abelian group is of order  $p^m$ , and of type  $1^m$ ,  $p$  being any prime number, there is a well-known formula which gives the total number of the subgroups directly. In particular, it can be seen from the following lists that among the groups whose orders are below 30 the abelian group of order 16 and of type  $1^4$  contains 65 subgroups, which is the largest number of subgroups contained in one of these groups. The generalized dihedral group of order 24 contains 52 subgroups, which is next to the largest number.

*List of the Groups and of the Numbers of Their Subgroups.*—Since a group of prime order contains no proper subgroup these orders are not included in the following list. The only other order below 30 for which there is no non-cyclic group is 15. It is known that a necessary and sufficient condition that there is only one group of a given composite order is that this order is the product of distinct prime numbers such that none of them diminished by unity is divisible by another. The number of the subgroups of such a group is equal to the number of these distinct prime numbers. In the following enumeration of groups only distinct abstract groups are considered while in the enumeration of the subgroups two subgroups are regarded as distinct unless each of them contains exactly the same operators as the other. For instance, the given group which contains 65 subgroups contains only three distinct abstract subgroups; viz., one of each of the orders 2, 4, 8. A similar enumeration with respect to abstract groups would not, in general, be possible, but it has frequently been employed in regard to the permutation groups of a given degree.

Exactly half of the 14 groups of order 16 contain more subgroups than operators. These are the dihedral and the hamiltonian groups of this order, each of which contains exactly 17 subgroups; the abelian groups of types  $2$ ,  $1^2$  and  $1^4$ , respectively, which contain 25 and 65 subgroups, respectively; the generalized dihedral group which contains the abelian group of type  $2,1$  and involves exactly 33 subgroups; the group whose commutator subgroup of order 2 is not found in one of its cyclic subgroups of order 4, and the group whose central is a cyclic subgroup of order 4 but which in-

volves no operator of a larger order. Each of these two groups contains exactly 21 subgroups. The study of the various types of subgroups, such as invariant subgroups and commutator subgroups, has thus far received much more attention than the enumeration of the subgroups, but this enumeration clearly also throws light on the properties of the groups concerned and hence it may be permanently useful just as the enumeration of the possible groups of low orders has been. Important early contributions to the latter enumeration were made by A. L. Cauchy (1789-1857), J. A. Serret (1819-1885), A. Cayley (1821-1895) and others.

#### LIST OF THE GROUPS AND OF THE NUMBERS OF THEIR SUBGROUPS

ORDERS	DESCRIPTION OF THE GROUPS OF THE SAME ORDERS	NUMBERS
4	Cyclic, non-cyclic	1, 3
6	Cyclic, non-cyclic	2, 4
8	Cyclic, octic, quaternion Type 2, 1; type 1 <sup>3</sup>	2, 8, 4 6, 14
9	Cyclic, non-cyclic	1, 4
10	Cyclic, non-cyclic	2, 6
12	Cyclic, tetrahedral, dihedral Dicyclic, non-cyclic abelian	4, 8, 14 6, 8
14	Cyclic, dihedral	2, 8
15	Cyclic	2
16	Cyclic, dicyclic, dihedral, hamiltonian Type 3, 1; type 2 <sup>2</sup> ; type 2, 1 <sup>2</sup> ; type 1 <sup>4</sup> Non-abelian conformal with type 3, 1 Operator of order 8 and its third powers Generalized dihedral involving type 2, 1 The commutator of order 2 is not a square Twelve operators of order 4, different squares Invariant maximal operator of order 4	3, 9, 17, 17 9, 13, 25, 65 9 13 33 21 13 21
18	Cyclic, non-cyclic abelian, dihedral Generalized dihedral, central order 3	4, 10, 14 26, 12
20	Cyclic, non-cyclic abelian, dihedral Dicyclic, metacyclic	4, 8, 20 8, 12
21	Cyclic, non-cyclic	2, 8
22	Cyclic, dihedral	2, 12
24	Direct products abelian Sylow subgroups Direct products, non-abelian orders 8 Non-twelve, direct product tetrahedral and 2 Symmetric, dihedral, dicyclic Generalized dihedral, generalized dicyclic Operator order 12 and its fifth powers Non-cyclic abelian 12, octic and symmetric 6	6, 14, 30 18, 10 13, 24 28, 32, 16 52, 24 24, 8 28
25	Cyclic, non-cyclic	1, 6
26	Cyclic, dihedral	2, 14
27	Type 3; type 2, 1; type 1 <sup>3</sup> Non-abelian conformal with 1 <sup>3</sup> , with 2, 1	2, 8, 26 17, 8
28	Cyclic, non-cyclic abelian, dihedral, dicyclic	4, 8, 26, 10

# A GENERAL THEOREM ON CONFORMAL MAPPING OF MULTIPLY CONNECTED DOMAINS

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1. *Introduction.*—In the theory of conformal mapping of  $k$ -fold connected domains the basic problem is to find types of domains  $B$  which depend on a finite number of parameters (essentially equal to the number of moduli) such that any  $k$ -fold connected domain is conformally equivalent to one of them. Such a class of domains  $B$  will be called a *class of normal domains*. Methods developed for the solution of Plateau's and Douglas' problem have opened a new approach to this mapping problem. The present note, which depends on previous publications,<sup>1</sup> shows on this basis that a very wide variety in the choice of normal domains is possible.

**THEOREM:** *The domains consisting of the exterior of  $k$  non-intersecting curves which are respectively similar under expansions and translations<sup>2</sup> to any  $k$  given convex analytic curves form a class of normal domains.*

*Proof.*—Let  $G$  be an arbitrary domain in the  $(x, y)$ -plane bounded by  $k$  non-intersecting Jordan curves  $\Gamma_j$ ,  $j = 1, 2, \dots, k$ . Without restricting the generality we may assume that  $G$  is bounded. It is required to map  $G$  conformally on a domain  $B$  of the  $(u, v)$ -plane with boundaries  $C_j$ ,  $j = 1, 2, \dots, k$ , of the type stated in the theorem. The mapping under consideration will be given by the functions  $x(u, v)$ ,  $y(u, v)$ , the components of a vector function  $\mathfrak{X}(u, v)$ . The function  $\mathfrak{X}(u, v)$  and the domain  $B$  are characterized as the solution of the following minimum problem: In a domain  $B$  of our class, consider vectors  $\mathfrak{X}(u, v)$  which are continuous in  $B + C$  with piecewise continuous derivatives in  $B$ , map the curves  $C_j$  continuously and monotonically on  $\Gamma_j$  and have a finite Dirichlet integral,

$$D[\mathfrak{X}] = \frac{1}{2} \int_B (\mathfrak{X}_u^2 + \mathfrak{X}_v^2) du dv;$$

we seek the absolute minimum of the Dirichlet integral with respect to the vectors  $\mathfrak{X}$  and the domains  $B$ .

That this variational problem has a solution  $\mathfrak{X}$ ,  $B$ , and that  $\mathfrak{X}$  is harmonic in  $B$ , has been proved in the papers [1], [2]. The only remaining point is to show that the variational conditions imply a conformal mapping of  $B$  on  $G$ ; this is equivalent to showing that the analytic function

$$\Phi(w) = (\mathfrak{X}_u - i\mathfrak{X}_v)^2 = \mathfrak{X}_u^2 - \mathfrak{X}_v^2 - 2i\mathfrak{X}_u\mathfrak{X}_v = \left(\frac{d\mathfrak{f}}{dw}\right)^2$$

vanishes identically, where  $\mathfrak{f}$  is the analytic vector function with  $\mathfrak{X}$  as its real part.

2. *Variational Conditions.*—It will be useful to consider variational conditions not only in the plane of the variable  $w = u + iv$  but also in an auxiliary plane of the complex variable  $\zeta = re^{i\theta}$ .<sup>3</sup> Let  $w = w(\zeta)$  be the analytic function that maps the exterior of the unit circle  $K$  of the  $\zeta$ -plane conformally on the exterior of the curve  $C_j$  of the  $w$ -plane so that the point at  $\infty$  goes into the point at  $\infty$ . We have

$$\Phi(w) = \frac{(d\mathfrak{f}/d\zeta)^2}{(dw/d\zeta)^2} = \frac{\Psi(\zeta)}{(dw/d\zeta)^2}. \quad (1)$$

The variation of the boundary representation of  $\mathfrak{X}$  in the  $\zeta$ -plane yields<sup>4</sup>

$$\Im[\zeta^2\Psi(\zeta)] = 0 \text{ on } K \quad (2)$$

where  $\Im$  denotes “imaginary part.” The variation of the position of  $C_j$  by parallel translations in the  $w$ -plane yields a condition for  $\Phi(w)$  which, when transformed into the  $\zeta$ -plane, becomes

$$\int_K \frac{\Psi(\zeta)}{dw/d\zeta} d\zeta = 0. \quad (3)$$

Finally, varying by an expansion in the  $w$ -plane, we get

$$\Im \int_K \frac{\Psi(\zeta)w(\zeta)}{dw/d\zeta} d\zeta = 0. \quad (4)$$

We will express the conditions (2), (3), (4) in terms of the parameter  $\theta$  of the unit circle  $K$ . Setting  $\zeta^2\Psi(\zeta) = f(\theta)$  on  $K$  and noting that there  $\frac{dw}{d\zeta} = \frac{du + idv}{i\zeta d\theta}$  where  $du, dv$  are differentials on  $C_j$ , we obtain

$$f(\theta) \text{ is real,} \quad (5)$$

$$\int_K g(\theta) \frac{du}{d\theta} d\theta = \int_K g(\theta) \frac{dv}{d\theta} d\theta = 0, \quad (6)$$

$$\int_K g(\theta) \left[ u \frac{dv}{d\theta} - v \frac{du}{d\theta} \right] d\theta = 0, \quad (7)$$

where  $g(\theta) = \frac{f(\theta)}{|dw/d\zeta|^2}$ .<sup>5</sup>



By virtue of (6), condition (7) can be extended to

$$\int_K g(\theta) \left[ u' \frac{dv'}{d\theta} - v' \frac{du'}{d\theta} \right] d\theta = 0 \quad (7')$$

where  $u' = u - u_0$ ,  $v' = v - v_0$  and  $u_0, v_0$  are any constants.

3.  $\Phi(w) \equiv 0$ .—As in [1], the proof that  $\Phi(w) \equiv 0$  follows by counting its zeros in  $B$ . We first show that  $f(\theta)$  has at least four zeros on  $K$ . Let  $(u_0, v_0)$  be a point interior to  $C_j$ ; then  $u' \frac{dv'}{d\theta} - v' \frac{du'}{d\theta} = u'^2 \frac{d}{d\theta} \left( \frac{v'}{u'} \right) \geq 0$  since  $C_j$  is convex. The condition (7') shows that  $g(\theta)$ , and therefore  $f(\theta)$ , must change sign. Thus,  $g(\theta)$  has at least two zeros  $\theta = \alpha$ ,  $\theta = \beta$  where  $g(\theta)$  changes sign.

We can determine three constants  $a, b, c$ , not all zero, so that the function

$$h(\theta) = -b \frac{du}{d\theta} + a \frac{dv}{d\theta} - c \left( u \frac{dv}{d\theta} - v \frac{du}{d\theta} \right) = \frac{du}{d\theta} (cv - b) - \frac{dv}{d\theta} (cu - a)$$

vanishes at  $\theta = \alpha$  and at  $\theta = \beta$ . This function  $h(\theta)$  is the outer product of the vectors  $\left[ c \frac{du}{d\theta}, c \frac{dv}{d\theta} \right]$  and  $\left[ u - \frac{a}{c}, v - \frac{b}{c} \right]$ , the former tangent to  $C_j$  at the point  $(u, v)$ , the latter on the line through  $(u, v)$  and  $\left( \frac{a}{c}, \frac{b}{c} \right)$ . Since  $C_j$  is convex, there are only two points  $(u, v)$  where this outer product vanishes; moreover,  $h(\theta)$  changes sign there since the sine of the angle between the two vectors mentioned above changes sign. Now the conditions (6), (7) yield  $\int_K g(\theta) \cdot h(\theta) d\theta = 0$ ; this shows that  $g(\theta)$  must change sign at other places besides  $\theta = \alpha$  and  $\theta = \beta$ , for otherwise  $g(\theta) \cdot h(\theta)$  would have constant sign. It follows that  $g(\theta)$ , and therefore  $f(\theta)$ , has at least four zeros on  $K$ .

The analytic function  $\Phi(w)$  has at least four zeros on  $C_j$  by virtue of (1) and the fact that  $\frac{dw}{d\zeta}$  is regular and different from zero on  $K$ . Furthermore,  $\Phi(w)$  is regular in  $B$  and has a zero of order at least four at  $\infty$ . For, in the neighborhood of  $\infty$ ,

$$\zeta = \mathfrak{A} + \frac{\mathfrak{B}}{w} + \frac{\mathfrak{C}}{w^2} + \dots,$$

$$\frac{d\zeta}{dw} = -\frac{\mathfrak{B}}{w^2} + \dots,$$

and 
$$\Phi(w) = \left( \frac{d\zeta}{dw} \right)^2 = \frac{\mathfrak{B}^2}{w^4} + \dots$$

If  $\Phi(w)$  were not identically zero, the number  $N$  of its zeros would be given by  $N = \sum_{j=1}^k N_j$  where the individual terms are

$$N_j = \frac{1}{2\pi i} \oint_{C_j} d \log \Phi(w) = \frac{1}{2\pi i} \oint_{K_j} \left[ d \log f(\theta) - d \log \zeta^2 \left( \frac{dw}{d\zeta} \right)^2 \right]$$

and  $K_j$  is the unit circle modified by arcs circumventing the zeros of  $f(\theta)$  on the boundary and protruding outside the unit circle. The sense of integration is such that the exterior of the unit circle remains on the left. Because  $f(\theta)$  has at least four zeros on the boundary, and  $\zeta^2 \left( \frac{dw}{d\zeta} \right)^2$  has a pole of the second order at  $\infty$ , we have  $N_j \leq -4(1/2) + 2 = 0$ . Thus  $N \leq 0$  contradicting  $N \geq 4$ , so that  $\Phi(w)$  must be identically zero. q. e. d.

4. *Concluding Remarks.*—It would have been sufficient to show that  $N \leq 3$  to arrive at a contradiction. This allows us to normalize the domain  $B$ . One such normalization is to keep  $C_1$  fixed in size and position and allow  $C_2$  to vary only by expanding with respect to an interior point. This would give four zeros on  $C_j$ ,  $j \neq 1, 2$ , two zeros on  $C_2$  and no information on  $C_1$ . Thus,  $N_1 \leq 2$ ,  $N_2 \leq 1$ ,  $N_j \leq 0$  for  $j \neq 1, 2$ , and finally  $N \leq 3$ . In this case,  $C_1$  need not be convex and  $C_2$  need only be star shaped.<sup>7</sup> Another type of normalization is to allow  $C_1, C_2, C_3$  to vary only by expanding with respect to an interior point of each.

Finally, it should be stated that the property of convexity was used in an essential way in the proof although no such restriction is needed in the existence proof for the minimum problem.<sup>8</sup> However, mapping theorems where certain non-convex curves appear have been established on the basis of Plateau's problem in the thesis of B. Manel, [4]. Also included in this thesis are cases where singularities appear on the boundaries of the normal domain.

<sup>1</sup> See the following papers:

[1] R. Courant, "Plateau's Problem and Dirichlet's Principle," *Ann. Math.*, **38**, 679–724 (1937).

[2] R. Courant, "Conformal Mapping of Multiply Connected Domains," *Duke Math. Jour.*, Oct., 814–823 (1939).

[3] R. Courant, "The Existence of Minimal Surfaces of Given Topological Structure," *Acta Math.*, **72**, 51–98 (1940).

See also,

[4] B. Manel, "Conformal Mapping of Multiply Connected Domains on the Basis of Plateau's Problem," doctoral thesis at New York University, June 1939, to be published soon.

Complete references to further literature, in particular the works of Douglas, are found in [3].

<sup>2</sup> I.e., under transformations of the form  $x' = ax + b$ ,  $y' = ay + c$ .

<sup>3</sup> This procedure is used for elliptical domains in [4].

<sup>4</sup> Variational conditions have been derived in [3].

<sup>5</sup>  $\frac{dw}{d\zeta}$  is regular and different from zero on  $K$ .

<sup>6</sup> If  $c = 0$ ,  $h(\theta)$  represents the outer product of the vector  $\left[ \frac{du}{d\theta}, \frac{dv}{d\theta} \right]$  and the fixed vector  $[-a, -b]$ ; the reasoning still applies.

<sup>7</sup> A curve  $C$  is star-shaped if there is a point such that any straight ray issuing from this point intersects  $C$  exactly once.

<sup>8</sup> It seems plausible that the question of uniqueness, which is not discussed in this paper, is connected with the condition of convexity.

## *RIBONUCLEIC ACIDS IN BOTH NUCLEUS AND CYTOPLASM, AND THE FUNCTION OF THE NUCLEOLUS*

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The material exchanges between nucleus and cytoplasm were studied actively in the early days of cytology, a study which reached its climax in the theory of "trophochromatin."<sup>1</sup> The methods used at that time were the biological stains which were then considered specific, but more detailed work showed that identity of staining reaction by no means implied identical substances. Particularly with the use of the Feulgen reaction for thymonucleic acid, the evidence for a direct transfer of chromosomal material to the cytoplasm broke down.<sup>2</sup> At the center of the discussion was the nucleolus, which in its staining properties resembled some of the cytoplasmic components, and in some cases appeared indeed to be extruded into the cytoplasm. With the recent advances in cytochemical technique, these problems can be studied more critically. The methods of ultra-violet spectrophotometry applied to the cytological material have been particularly useful in the analysis of the occurrence and distribution of the nucleic acids in the cell. As has been pointed out in detail elsewhere,<sup>3</sup> the absorption spectra of the nucleic acids are sufficiently striking—the maximum lying at 2600 Å in the middle ultra-violet, due to the conjugated double bonds of the constituent pyrimidine rings—so that the course of the absorption curves of biological structures can give evidence of the presence in them of these substances.

As is well known the nucleic acids are differentiated into two groups according to the type of carbohydrate:<sup>4</sup> the desoxy ribose, of which the thymonucleic acid characterizing the chromosomes is the type; and the

ribose nucleic acids, to which the nucleic acid of yeast belongs. The absorption spectra, being determined by the nitrogenous constituents, do not differentiate between these two groups; but in conjunction with other tests the location of any of these substances in the cell may be determined by their use. In this way it was shown that the ribose nucleic acids are found in the cytoplasm of rapidly growing tissues in characteristically high amounts.<sup>5</sup> The data to be presented show that these substances are also present in the nucleolus and in high concentration around the nuclear membrane. Thus one of the rôles of the nucleus in cellular syntheses concerns the cytoplasmic nucleic acids, a discussion of which is of obvious relevance to the mode of action of genes.

Our measurements were made upon the egg of the sea urchin, *Psammechinus miliaris*, the spinach root tip periblem cell and the cells of the *Drosophila melanogaster* salivary gland. In the sea urchin we have an egg in which there are no elaborate systems of nutrition but the brunt of the syntheses is born by the egg itself, without any appreciable evidence of endomitosis in the nucleus. The root tip of *Spinacia*, on the other hand, shows clearly the occurrence of endomitosis, Gentcheff and Gustafsson<sup>6</sup> having recently worked over the double reproduction of the chromosomes in the periblem cells of this form. Finally the salivary gland cell allows the study of composition of the nucleolus in relation to the giant chromosomes and, more especially in *Drosophila*, different genetic types may be compared.

For these measurements preparations were made of cells fixed in a lanthanum acetate-acetic acid mixture, pressed between quartz cover slip and slide, with glycerine then allowed to diffuse under the cover slip. In order to obtain such isolated cells from the spinach root tip it was necessary to macerate the root in 45% acetic acid for several days, after which time the individual cells were teased apart, and the precipitation completed in the lanthanum acetate-acetic acid mixture.

The technique of the absorption measurements has been described in detail elsewhere.<sup>7</sup> The suitable places chosen in the relatively homogeneous nucleoli are centered in the ultra-violet microscope illuminated with monochromatic light dispersed by a mirror monochromator from a high-pressure, water-cooled mercury lamp. The light is directed onto the opening of a diaphragm in front of a photoelectric cell by means of an adjustable quartz prism and the current measured by a string electrometer of the Lutz-Edelman type. The object is then moved by means of a precision mechanical stage, until a neighboring free space is at the center of the field. Now by means of a variable rotating sector the light is cut down until the electrometer reading reaches the same value as the reading when the object was in the field. The percentage of light removed by the sector is then equal to the absorption by the object. The movement of the mechanical stage is sufficiently accurate in the case of relatively large objects like the nucleoli, so that the absorption measurements made at the different wave-lengths are all made at the same place in the object.

The absorption spectra of different regions within the nucleus and cytoplasm of the sea urchin egg are given in figure 1. The course of two of the four curves is evidently determined by the contained nucleic acids. The absorption spectrum of the nucleolus shows a typical absorption maximum at 2600 Å, with a subsidiary hump around 2800 Å, indicating the probable presence of proteins containing the aromatic amino acids such as tyrosine

and tryptophane. A similar absorption curve is given by the cytoplasm around the nuclear membrane, with the difference that there is considerably more absorption due to the protein. The curve for the more peripheral cytoplasm is quite different; there is a high absorption in the short wave-lengths which sinks to a hump around 2600 Å beyond which there is a second lower plateau around 2800 Å. Finally to complete the picture, the

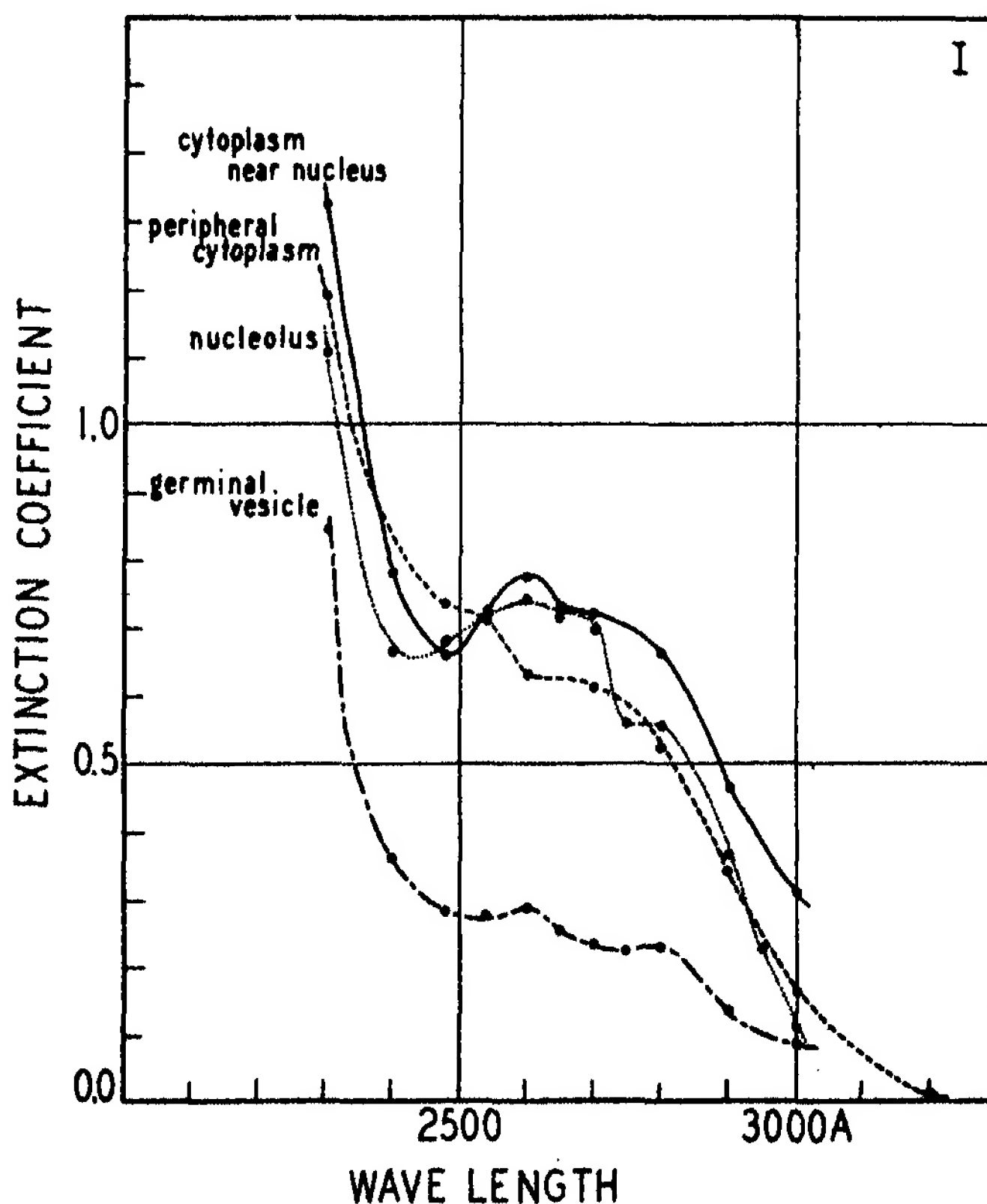


FIGURE 1

Ultra-violet absorption spectra of different parts of an ovarian egg of the sea urchin, *Psammechinus miliaris*. Measurements taken photoelectrically; optical conditions: objective 2.5, ocular 5 ×.

absorption spectrum of the nuclear sap and extended chromosomes of the germinal vesicle shows a slight rise in the nucleic acid region and a plateau in the region of the "protein" band. Towards the shorter wave-lengths the rise is more rapid than that exhibited by the other curves, the ratio of the absorption at 2300 to the absorption at 2800 Å being 3.7 for the germinal vesicle and only 2.3 for the peripheral cytoplasm. The absorption

curves for the various proteins are largely determined by the constituent amino acids;<sup>3,8</sup> and the protamines first isolated from fish sperm by Miescher contain only amino acids which give a high non-specific absorption at the short wave-lengths.<sup>3</sup> These differences observed in the absorption curves may perhaps be attributed to the presence in the germinal vesicle of the sea urchin of protamine like proteins.

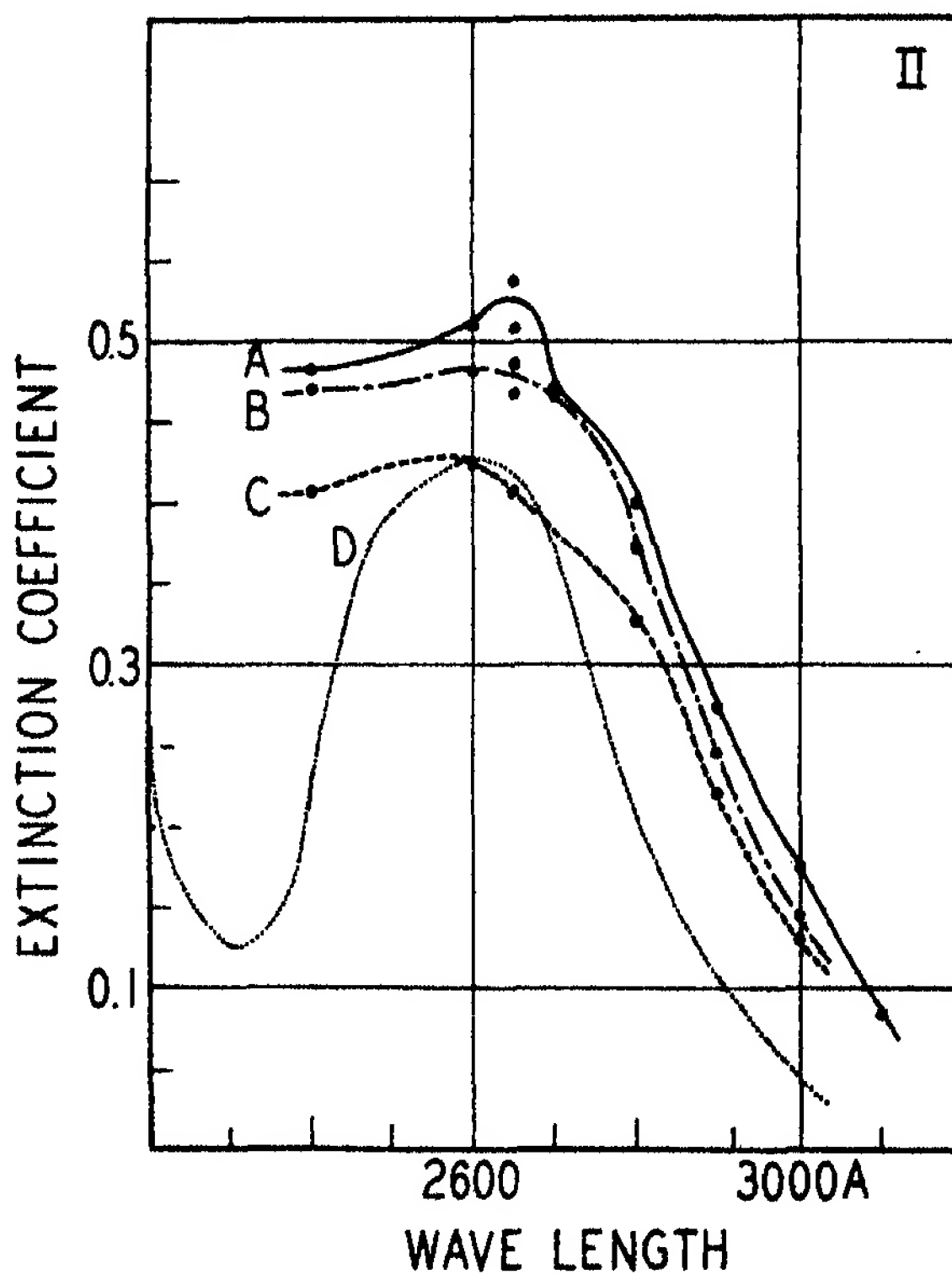


FIGURE 2

Ultra-violet absorption spectra of cytoplasm and nucleolus of *Spinacia oleracea*, var. *Hertha*. Curves A and B, nucleoli; C, cytoplasm of the same cell as B; D, yeast nucleic acid in concentration corresponding to absorption of C at 2600. Optical conditions, objective 2.5, ocular 10 X, condenser 1.25.

According to Brachet<sup>9</sup> the only Feulgen-positive structures in the sea urchin egg are the chromosome threads, which presumably account for the slight elevation of the absorption in the germinal vesicle in the region of the nucleic acid band. Thus the absorption data agree with the chemical data in showing that the major part of the nucleic acids of the sea urchin



egg belong to the ribonucleic acids. (Only the pentose nature of the carbohydrate has actually been determined for the sea urchin nucleic acid;<sup>9</sup> but all of the nucleic acids so far analyzed in detail are either ribose or desoxy ribose.) Moreover this ribonucleic acid is not only present in the nucleolus but its maximum cytoplasmic concentration is close to the nuclear membrane.

For the spinach cells similar measurements were made of nucleoli and also of cytoplasm for one of the cells (Fig. 2). The cells chosen for measurement were in early prophase, following a double reproduction. In these cells there is no striking accumulation of absorbing substances around the nuclear membrane. Both nucleolus and cytoplasm have their absorption maximum at 2600; the chief component may then be ascribed to the pyrimidine band of the nucleic acids. Comparison with the absorption spectrum of yeast nucleic acid in concentration sufficient to give the absorption of the cytoplasm at 2600 Å ( $5.3 \times 10^{-7}$  mg./ml.) shows, however, that other absorbing substances, presumably as in the case of the sea urchin protein in nature, are present. Only the chromosomes give the Feulgen reaction; hence these nucleic acids in the nucleolus and cytoplasm of spinach are not desoxyribose acids. In these cells, presumably secretory in function, there is again the association of plentiful ribonucleic acid with active growth.

The nucleoli of the salivary gland in *Drosophila melanogaster* are Feulgen negative, and not so pronouncedly basophilic as the two cases previously discussed. They are typical "plasmosomes," with considerable variation in their cytological appearance, stainability, etc. The absorption spectra of the cytoplasm in the salivary gland will be discussed elsewhere; they vary with the stage of development of the gland, beginning with a high nucleic acid concentration, which decreases as the gland matures. Accumulations of absorbing material are sometimes seen around the nuclear membrane.

In figure 3, III, the absorption spectra are given of nucleoli from a single gland of a male larva from the *Swedish B* stock (one of the standard "wild" stocks). The curves belong to the same family, the differences being minor ones in height, due possibly in some cases to variation in thickness after smearing, as well as to slight differences in composition. They all show a high absorption at the short wave-lengths, a hump around the nucleic acid maximum, and another lower level around the tyrosine-tryptophane band of the proteins. This nucleolus contains therefore a nucleoprotein with a lower percentage of nucleic acid than the others tested, so that the absorption of the proteins themselves plays a dominant rôle in the determination of the characteristics of the curve.

The absorption spectra of nucleoli in two sister females were also measured (Fig. 3, IV). In these nucleoli the nucleic acid maximum is still

further masked; the curve can be fitted by that of a nucleoprotein containing about 3.5% nucleic acid, the protein having the absorption characteristics of serum albumin. The curve for the male necessitates a different type of protein and about twice the percentage of nucleic acid. It is indicated, therefore, that the composition of the nucleoli is not a constant character but may vary within the species according to the genetic composition of the individual. This problem will be further discussed in the succeeding paper.

*Discussion.*—These three cases are, we believe, representative of various types of nucleoli. "True" nucleoli take basic dyes, are variable from group to group and give a negative Feulgen reaction (except for a few isolated cases<sup>2</sup> and for the so-called chromatic inclusions which are of interest in the problem of nucleolar synthesis). These characteristics, as well as their diverse behavior in digestion with enzymes,<sup>10</sup> are adequately explained by the assumption that the nucleoli are composed of ribonucleoproteins, containing varying percentages of nucleic acid. Indeed, since this work was completed Brachet<sup>11</sup> has shown that the ribonuclease of Dubos deprives Amphibian nucleoli and cytoplasm of their capacity to take the pyronin of the *Unna* methyl green-pyronin mixture, which would indicate that these nucleoli and cytoplasms also contain ribonucleic acids which are responsible for their basophily, in agreement with our view. The "specific" nucleolar stain of Semmens and Bhaduri<sup>12</sup> gives evidence of another sort. The technique involves the Feulgen procedure, after which the tissue is left in 5% sodium carbonate solution for an hour, to be stained in fast green thereafter and differentiated in 70% alcohol saturated with the carbonate. Under these conditions the fast green stain remains in the nucleoli. It seems likely that the ribonucleic acids are dissolved in the carbonate solution, since a similar procedure can be used for their extraction when it is desired to separate them from the thymonucleic acids (Delaporte).<sup>13</sup> The reaction to the stain after the solution of the ribonucleic acids would then be due to the presence of highly basic proteins (protamines, histones) in the nucleolus. There are no detailed experiments on the reactions of ribonucleoproteins with dyes, such as those carried out on the thymonucleic acids.<sup>14</sup> It seems likely that the variations in the basophily of the nucleoli may be explained by the old hypothesis of variation in the nucleic acid content. Parenthetically, it may be remarked that claims of the presence of lipoids in the nucleolus are based mainly on the destruction of the structure by "lipoid solvents." Since these are also protein denaturing agents, the use of such methods is not instructive. There is to date no good evidence of lipoids in the nucleolus.

The rôle of the nucleic acid compounds, particularly the nucleoproteins, in the general metabolism of the cell occupies increasing prominence, above all in the processes of synthesis accompanying growth and secretion

The enzymatic rôle of nucleoproteins in biological oxidations has been shown by the work of Warburg<sup>15</sup> and others; the viruses as they are purified and analyzed one by one fall consistently into the same group;<sup>16</sup> and cases are gathering in which the nucleoproteins act as growth factors. The cytochemical approach traces the correlation between the occurrence of changes in the composition of the parts of the cell itself and the processes going on at the time. We have already reported for a number of cases that cells in which active synthesis or growth is occurring are rich in the cytoplasmic ribonucleic acid compounds.<sup>5</sup> The present data permit

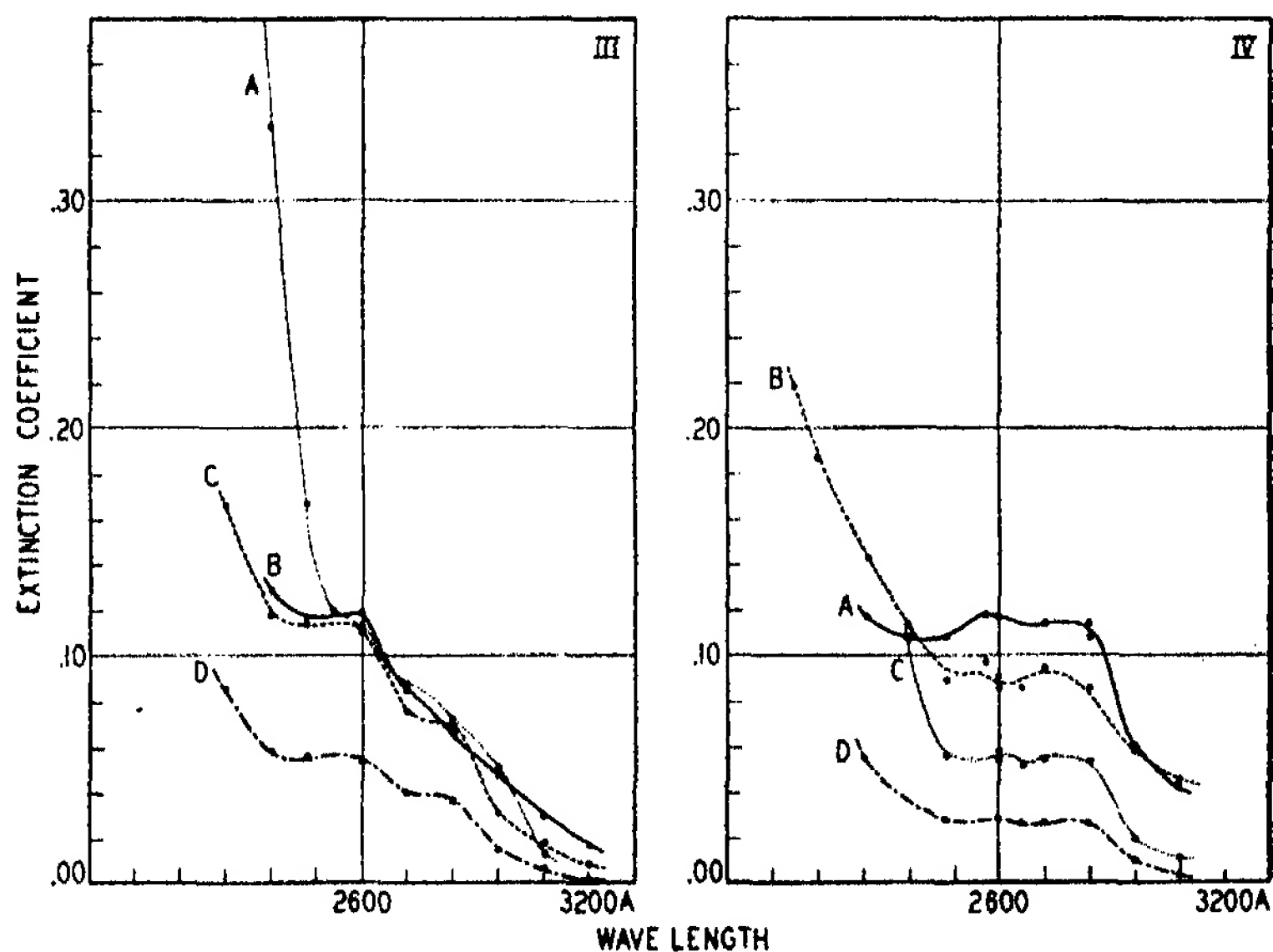


FIGURE 3

Ultra-violet absorption spectra of male (III) and female (IV) nucleoli from the Swedish B stock raised at 25 degrees. The male nucleoli are all from the same larva; among the females, A, B and C are from the same individual, D from another. Optical conditions: objective 2.5, ocular 10 X.

the view to be discussed that these substances have their origin in the synthetic activity of the nucleus.

In the sea urchin eggs the nucleolus has a high content of ribonucleic acid compounds, the nuclear sap a low content, and there is an accumulation of these substances in the cytoplasm around the nuclear membrane. There is thus no evidence for a direct transport of these substances from the nucleolus to the cytoplasm before a breakdown of the germinal vesicle. But they already are present in the cytoplasm at this time; it would seem, therefore, from the presence of a gradient from the nuclear membrane peripherally that the synthesis occurs at that locus. In this way the syn-

thesis of nuclear products influencing cytoplasmic activity would take place at a surface which by its very nature is the joint product of both.

It should be noted that such a gradient around the nuclear membrane will not necessarily be apparent unless the rate of synthesis exceeds the rate of transport. Thus such accumulations of absorbing material around the nuclear membrane do not occur noticeably in the root tip of either *Allium* or *Spinacia*. Nor do they occur around cleavage nuclei. But according to Brachet<sup>9</sup> the ribose nucleic acids are depleted during the early development of various marine eggs, while the thymonucleic acid content rises correspondingly. Thus during the cleavages in which very rapid division proceeds the ribose nucleic acid content decreases, and it would seem that here the cytoplasm may somehow serve as a direct source of precursors for the chromosomes. The data do not, however, support Painter's<sup>17</sup> view that the chromosomes endomitotically multiplied during oögenesis themselves serve to provide these cytoplasmic precursors. As has already been shown here for the sea urchin, and the same is true both in *Drosophila* and *Musca*, the cytoplasmic synthesis takes place before the breakdown of the nuclei.

The rôle of the nucleoli in the synthesis of the cytoplasmic nucleic acids is difficult to evaluate. The generalizations about nucleoli that were made very early<sup>18</sup> are still applicable and are suggestive when considered in terms of the ribonucleic acid synthesis. Nucleoli of cleavage cells are in general small and inconspicuous; nucleoli from cells in which active synthesis is proceeding (gland cells, the growing oöcyte) are generally large and complex; disappearance or breakdown of the nucleoli in mitosis is correlated with the development of the chromosomes. In some cases extrusion of nucleolar fragments into the cytoplasm has been described. In recent years it has been established that the nucleoli are produced at specific chromosomal loci in heterochromatin.<sup>19</sup> The various data can be accommodated under the view that these chromosome regions are especially concerned with the ribonucleic acid metabolism of the cell. Evidence for this view is given in the succeeding paper. Here we need only say that the activity of the nucleoli is closely associated with an intense synthesis of the cytoplasmic ribonucleic acids. And, as has already been pointed out, the association of these substances with the processes of synthesis seems well enough established so that their study from the point of view of the nucleocytoplasmic relationships may provide some insight into the mode of action of the genes.

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## THE GENETIC CONTROL OF NUCLEOLAR COMPOSITION

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In a sense the study of the nucleolus occupies a key position in the analysis of the inter-relations of the nucleus and the cytoplasm. It is a structure within the nucleus, resembling the cytoplasmic structures in the type of nucleic acid it contains; at the same time, like other genetic effects, it is related to a definite locus in the chromosomes. Moreover, as has already been discussed in the previous paper, its function appears somehow to be connected with the synthetic activities of the cytoplasm. This being the case, the effects of genetic changes in the nucleolar regions upon the composition of the nucleolus should give some insight into the possible uses of this structure for the analysis of the problems associated with the action of the genes. Viewed from another aspect, changes in the composi-



tion of the nucleolus due to changes in the chromosomes should allow an additional attack on the relation of the thymonucleic and the ribonucleic acids. Evidence is here presented that the characteristics of the nucleolar nucleoproteins in *Drosophila melanogaster* are subject to change by genetic factors.

The nucleolus in *Drosophila melanogaster* has been shown to be associated with the heterochromatin of the *X* and the *Y* chromosomes.<sup>1</sup> A more detailed analysis of the available data gathered by different workers on its locus within the heterochromatic regions in the salivary gland chromosomes indicates that the locus of the nucleolar region in the *X* heterochromatin is close to, if not identical with, that of the locus for the large block of thymonucleoprotein in the metaphase chromosome.<sup>2</sup> Whether the nucleolar region of the *Y* is identical with that of the *X* cannot be determined at present. In the preceding paper we have shown the existence of a sexual difference in the nucleolar composition; this difference may be due to a difference between *X* and *Y*, the male containing both an *X* and a *Y* chromosome, the female containing two *X*'s. In the following, measurements of the absorption spectra of the nucleoli of males and females containing various chromosome rearrangements are described. The data are exploratory in nature, and having been collected upon preparations used for other purposes as well do not form as simple and complete a series as could have been planned for the special study of the nucleolus.

Two *X* chromosome rearrangements were used, one of which, symbolized as  $X^c$ ,<sup>3</sup> involves a break in heterochromatin within the nucleolar region. The other rearrangement,  $X^{c2}$ , also has one break in the heterochromatic region, but just outside the nucleolar region. Both of these are so-called "closed" or ring chromosomes. They are the result of translocation between the members of a pair of attached *X* chromosomes,<sup>4</sup> being deficient for a very small part of the tip of the *X* chromosome. Each contains a duplication of the heterochromatin from the point of rearrangement to the spindle attachment region. Thus for  $X^c$  the duplication extends from the nucleolar region to the spindle attachment; but  $X^{c2}$  contains a duplication for all of the *X* heterochromatin. Hence the studies of these chromosomes involve simultaneously the effects of rearrangement and of duplication. For the *Y* chromosome, the rearrangement studied is of a simpler nature, being an intercalation into the *Y* of a part of the second chromosome.<sup>5</sup> In an attempt to test the effect of heterochromatin other than that around the nucleolar regions, measurements were made of a rearrangement involving the exchange of part of the *X* chromosome for most of the small fourth chromosome, with the point of rearrangement in chromosome four being in heterochromatin, and that in *X* at the section of Bridges' map  $3F_1$ .<sup>6</sup> The technique applied has already been described in the previous paper.

The absorption curves for the male nucleoli are shown in figures 1A-E, IID and IVD-E. Those for the *Swedish B* (the "wild type" stock)



male have already been presented in the preceding paper. In each case where duplicate measurements are available they are of different nucleoli from the same individual. It is evident that the different types give

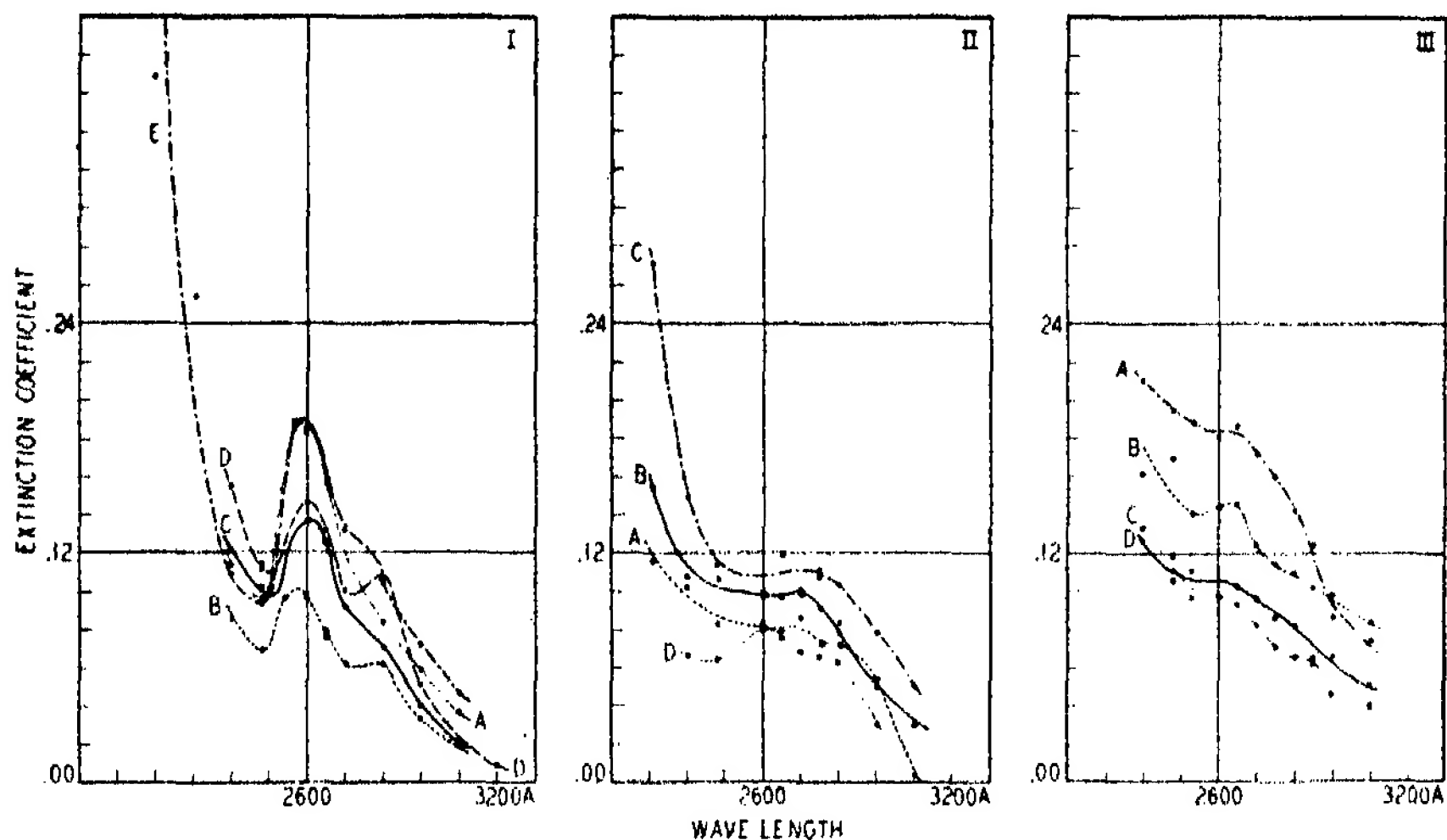


FIGURE 1

I. Absorption spectra of nucleoli from a larva heterozygous for the Y-2 translocation  $G$ , and the mutant genes black, light and brown. Culture raised at 20-22°C. Optical conditions for this and the other measurements here reported: objective, 2.5 mm.; ocular 10 X, condenser 1.25 mm., condenser diaphragm 6.

II. Absorption spectra of nucleoli:  $A, B, C$ , from a female larva,  $X^{cs}/y w dm$ ;  $D$ , from a male larva carrying  $X^{cs}$  (25°C.).

III. Absorption spectra of nucleoli from homozygous  $X^{cs}$  female larvae (25°C.).

IV. Absorption spectra of nucleoli:  $A, B, C$ , from a female larva heterozygous for  $w^{Ds}/y X^c$  (raised at 16°C.);  $D, E$ , from a male carrying  $y X^c$  raised at 25°C.

V. Absorption spectra of nucleoli:  $A, B$ , from a female larva  $w^{Ds}/y w dm$  raised at 18°C.;  $C$ , the same at 25°C.;  $D$ , from a female larva  $w^{Ds}/y w dm$ ;  $Y$  raised at 18°C.

characteristically different groups of curves, where the data are adequate to permit a judgment. The nucleoli of the Y-2 translocation male have absorption spectra that show a maximum around 2600 Å, with a hump at a

lower level corresponding to the band around 2800 of tyrosine and tryptophane. The males containing the closed  $X$  chromosomes show a different type of curve, with the 2600 peak more submerged in the "protein" absorption. A distinction between the two closed  $X$ 's is evident, although the scanty data for  $X^{c2}$  make caution necessary. In the wild type male, the 2600 maximum is present only as a hump in the curve, similar to that at 2800. The differences might be interpreted as due either to a decrease in the amount of protein in the different types containing rearrangements, as compared with the wild type, or, conversely, there could be an increased amount of nucleic acid. In order to determine this point adequately, measurements of the total amount of substance are necessary. As between the *Swedish B* and the  $T(Y-2)G$  males, the difference lies in the absorption at the short wave-lengths, since the ratios of the absorption at 2600 to that at 2800 are sensibly identical in the two cases (1.6 and 1.7). The ratio is similar for the  $X^c$  male (1.5), but the  $X^{c2}$  male is distinctly lower (1.3). It is thus possible that the increase in heterochromatin gives an increase of both nucleic acid and protein, while the effect of the rearrangement is to decrease the amount of protein formed. In such case the intermediate character of the  $X^c$  curve as compared with the wild type and the  $Y$ -translocation curves would be the result of a position effect on the  $X$  nucleolar region plus the effect of a duplication; in the  $Y$ -translocation we observe the position effect itself and in the  $X^{c2}$  male the effect of the duplication itself. These interpretations must, however, be regarded as provisional at present.

In the female series the data (Fig. 1, II (A-C); III) permit the comparison of the wild type from the preceding paper and  $X^{c2}$  with the heterozygous  $X^{c2}$  female. The ratios of the absorptions at 2600 and at 2800 are again instructive. For the  $X^{c2}$  female the ratio is 1.28; for the wild type it is 1.07; and for the heterozygote, 1.08. Evidently the effect of the closed  $X$  in heterozygous condition in the female is less marked than it is in the male, although even in this case a comparison of the ratios at 2650/2800 shows a slight effect:  $X^{c2}$ , 1.29;  $X^{c2}/ywdm$ , 1.15; *Swedish B*, 1.03. Without a more complete analysis of these curves into their component absorbing substances, and, as said above, a knowledge of the total absorption of the nucleoli, only tentative conclusions can be drawn. Apparently in this case as in the male, the duplication in the  $X^{c2}$  increases both the nucleic acid and the protein absorption. The question of the dominance of the normal nucleolar composition in the heterozygote where two differently produced nucleoli are fused is more complex. It brings up again the difference between the nucleolar composition of the sexes. As previously pointed out, this difference may be the consequence of the presence of the  $Y$  chromosome in the male, or it may be the result of the general difference of genic balance as between the sexes. A sexual differ-

ence similar to that found in the *Swedish B* measurements is found in the male and female  $X^{c2}$ , with the protein absorption again more marked in the female. If the effect of the  $X^{c2}$  be assumed to be due to a duplication of heterochromatin including the nucleolar region, the results fall into line with the behavior of duplications for  $X$  chromosome regions in *Drosophila* in general: the effect, that is, the difference from the wild type, is greater in the male than in the female for a duplication of any given part of the  $X$ . It is of interest that the complicated relations of genic balance should be met with in intra-nuclear characters; McClintock has already described a similar case in the behavior of the nucleolar region of *Zea mays*.

For the analysis of such possible genic balance relations, study of the effects of heterochromatic regions other than the nucleolar region itself should be of value. To this end measurements were made of the nucleoli in individuals heterozygous for the  $X$ -4 translocation (symbolized  $w^{pD3}$ ) previously referred to, and for the same chromosome as that used in the heterozygote for  $X^{c2}$ , containing the mutant genes yellow, white and diminutive; a measurement was also made of a similar heterozygote containing an extra  $Y$  chromosome. The data (Fig. 1, V) give a curve of marked nucleic acid character, with an evident effect of the extra  $Y$  chromosome in accentuating the maximum at 2600. These preparations, however, were made (in connection with studies of the variegated types correlated with rearrangements of this kind) from larvae raised at 18 degrees. A check measurement of a control raised at 25 degrees, and therefore directly comparable to the closed  $X$  heterozygote, shows a flatter curve. The absorption ratio for 2600/2800 is only 1.19, as compared with the ratio for the 18-degree series of 1.37. It appears, thus, that there is an increase in the ratio of nucleic acid to protein at the lower temperatures, perhaps similar in nature to the effects of rearrangement in the nucleolar regions. There seems still, however, to be an effect of the  $X$ -4 rearrangement on nucleolar composition; for even at 25 degrees the 2600/2800 ratio (1.19) is higher than the value of 1.08 found in the  $X^{c2}/y w dm$  heterozygote. The rearrangement here discussed also has the effect of inducing variegation for characters due to genes located in the regions transposed to heterochromatin; these changes have been postulated on the basis of changes seen in the salivary gland chromosomes to result from changes in the nucleoprotein ratio of the genes themselves. Should the present indication of an effect on the composition of the nucleolus be substantiated, evidence can be obtained for the interrelations of nucleolar and chromosomal composition. It would follow that in addition to the chromosomal effects of these rearrangements there is also an effect of heterochromatin on the nucleoprotein metabolism of the nucleolus.

The effect of the  $X$ -4 translocation on the nucleolus must be regarded as dominant, since it is manifested in the heterozygous female. It contrasts

with the effect of  $X^{c2}$  in this regard. We took the occasion to make measurements of heterozygotes for the  $X$ -4 rearrangement and for  $X^c$ ; it seemed possible that the more pronounced departure of  $X^c$  from the normal in the male might show in the heterozygous female as well. These heterozygotes

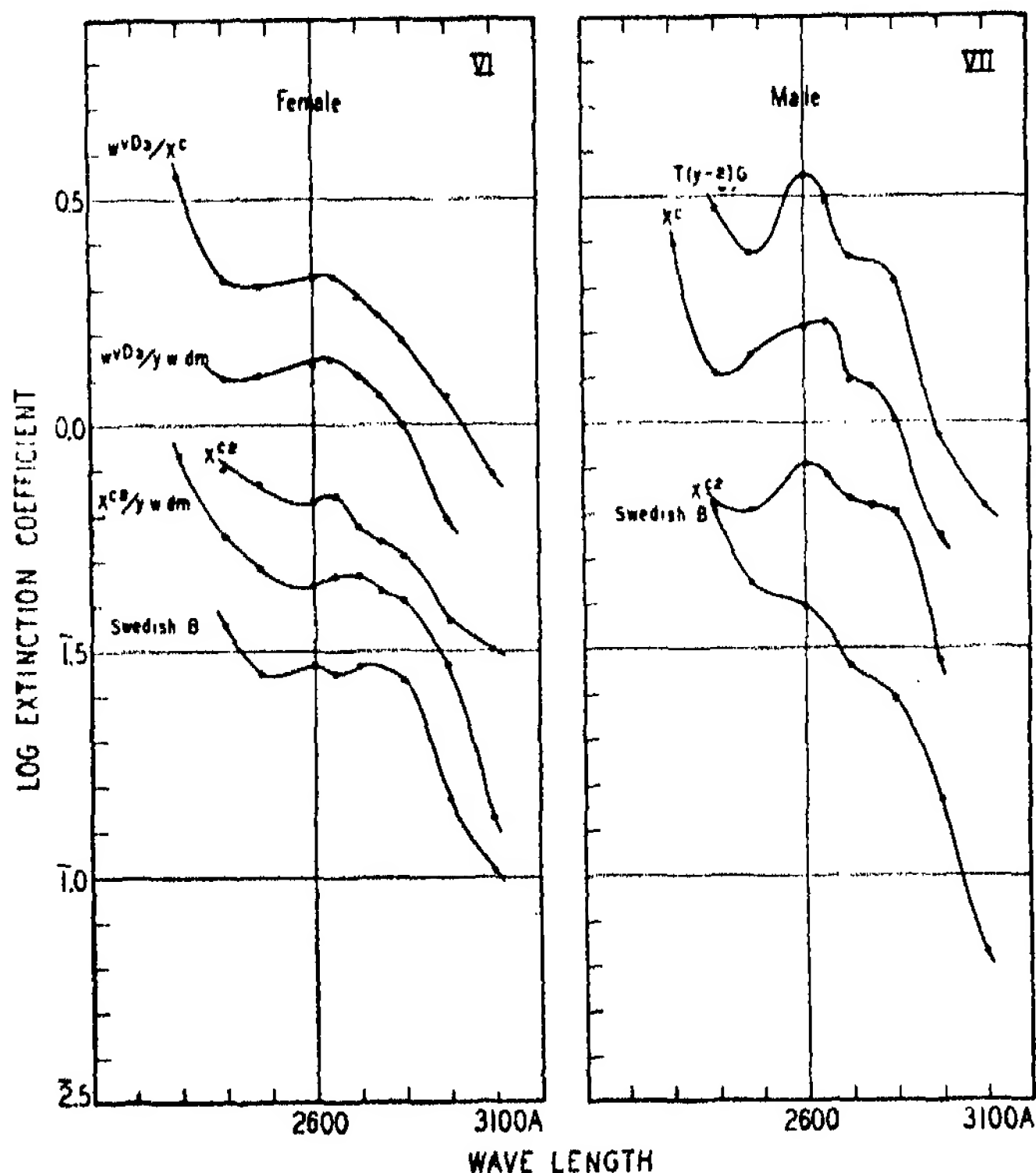


FIGURE 2

The data of the previous figures are summed for each type, and the logarithms of the extinction coefficient are plotted. For purposes of clarity they are placed at convenient distances on the scale of ordinates. The data for the *Swedish B* males and females have been summed, and the logarithms of the sums plotted directly. To the logarithms of the other sums the following have been added before plotting: females,  $X^{c2}/y w dm$ , 0.6;  $X^{c2}$ , 0.1;  $w^{vD3}/y X^c$ , 0.6; males,  $X^{c2}$ , 1.0;  $X^c$ , 0.9;  $T(Y-2)G$ , 0.8.

were raised at 16 degrees instead of 18, as were the other heterozygotes for this rearrangement. But in any case, as the 2600/2800 absorption ratios calculated from the data (Fig. 1, IV, A, B and C) show, there is no striking difference from the  $w^{vD3}/y w dm$  heterozygote. In the former case they are 1.41, 1.46 and 1.32, as compared with 1.42, 1.31 in the latter case.

*Discussion.*—A comparison of the different sets of data is made in figure 2. For each type the logarithms of the sums of the extinction coefficients of the individual nucleoli are plotted against the wave-length. In this way curves are obtained whose shape is independent of variations in concentration or thickness of the absorbing layer.<sup>7</sup> They are dependent solely on the nature of the constituent absorbing substances. Thus the logarithmic plots of the sums of the extinction coefficients for all the nucleoli of a given type give an estimate of the nucleolar composition such as would be obtained by an analysis of the composition of a mixture of all the nucleoli for each type. The different types can then be compared since nucleoli of like composition should give curves which are superposable each upon the other. It is evident that this is not the case for any of the types so plotted. The relations previously discussed emerge with especial clarity; the nucleic acid protein ratio is increased in the types with the rearrangements, as the height of the 2600 band shows. The males have in general a more pronounced maximum in the cases where direct comparison can be made. The females have a gentler group of curves, in which the protein absorption is more influential. But each of the genotypes has its own characteristic curve.

We have discussed the differences in the absorption curves as due to genetic differences. The objection might be raised that within an individual the data are consistent, but that random non-genetic differences between individuals could account for the differences observed. This seems unlikely, since the seriation of the types is self-consistent, and the agreement between such types as the  $X^{c2}/y w dm$  and the *Swedish B* female is so close as to permit the discussion of dominance even though the stocks are unrelated. Also, as is discussed below, the staining reactions of the nucleoli run parallel to our measurements. It follows then that the analysis of these changes in nucleolar composition is an analysis of the function of the genes in the formation and behavior of the nucleolus.

The "nucleolar genes" are located in the heterochromatic regions, and they must therefore partake of the properties of those regions, if indeed they are not responsible for some of them. Heterochromatic regions have the capacity (1) to form large amounts of thymonucleic acid (or, better perhaps, thymonucleoprotein) in the chromosomes themselves; (2) to form<sup>8</sup> or affect the composition of the nucleoli; (3) to affect the characteristics of neighboring regions translocated to them in such a way as to change the developmental effects of these regions in somatic cells<sup>9,10</sup>; (4) to affect the content of the ribonucleic acids in the egg cytoplasm of *Drosophila*.<sup>6</sup> Only the first and second of these characteristics are known for organisms other than *Drosophila melanogaster*, and they appear to be quite general properties. We are not here concerned with the specific cytological characteristics such as the property of indiscriminate synapsis, and the



type of chromomere formed by these regions in the giant chromosomes.

It would be premature to attempt a detailed correlation of these properties. In outline, however, they seem all to be different aspects of the nucleic acid metabolism of the cell. For the nucleolus, the evidence here given permits the further specification that the ribonucleoprotein metabolism is controlled by these regions. Thus the connection becomes close between a region of the chromosome which produces much thymonucleic acid and a structure composed of ribonucleoprotein. How direct this relation is cannot be told, for in *Zea mays*<sup>9</sup> the smaller portion of the nucleolar region forms the larger nucleolus and the presence of an additional nucleolar region has no effect on nucleolar size. On the other hand in *Solanum*, M. M. Lesley<sup>11</sup> has shown a quantitative correspondence between the size of the nucleolus and the size of the nucleolar region, in a series of variants. Failing measurements of the composition of these nucleoli to show that the differences are merely quantitative, definite statements are precarious. It seems likely, however, that the difference of behavior between *Zea* and *Solanum* is another example of the difficulties attendant on the use of the effects of deficiency or duplication of a chromosome section in the analysis of the effects of that section. For the composition and size of the nucleolus are dependent on the general genic balance, and only when the nucleolar region itself is the limiting factor in nucleolar production can we expect to see changes in the characteristics of the nucleolus due to it. Indeed McClintock has shown in *Zea* that other chromosome regions very easily take over the rôle of nucleolar synthesis; it is to be questioned whether this would so easily occur in *Solanum*.

There are wide variations in the behavior and the staining reactions of the nucleoli of even closely related species. The present results are particularly relevant to the variations described by Bauer<sup>12</sup> in the Chironomids in which some species show in aceto-carmin preparations a well-organized deeply staining nucleolus, others a light and diffuse type. In the present series in *Drosophila* similar variations are encountered, and indeed were the origin of this investigation. The parallel between the intensity of stain, the compactness of the nucleolus and the prominence of the nucleic acid band is rather good; in other words, the structural qualities of the nucleoli seem to be associated with their nucleic acid content. This indicates that the ribonucleic acids as well as the thymonucleic acids have a rôle in structure formation of the sort emphasized by Hammarsten.<sup>13</sup> In this way the genotypic control of the structural characteristics of the nucleolus may be mediated by changes in its nucleic acid content.

The heterochromatic regions are commonly known as genetically "inert" because where tests have been made no phenotypic changes are detected in the presence of duplications or deficiencies. It is evident from the data reported and discussed here and elsewhere that the "inert" char-



acter is factitious. Actually the situation is that of a system in which all components are present in excess, similar to that discussed in connection with the nucleolar region in *Zea*. This is not surprising, if as we have suggested in the previous paper the ribonucleic acid compounds are influential in a variety of cellular syntheses. On this view the heterochromatic regions are particularly concerned with the synthesis of the nucleic acids; in the chromosomes their activity is manifest in the blocks of thymonucleoprotein they produce; in the nucleolus and the cytoplasm they produce the ribonucleic acid compounds. In the formation and development of the nucleolus it is possible that we have, so to speak, a pattern of gene function; the ribonucleoprotein is formed at telophase, and in endomitotic nuclei grows parallel with the growth of the chromosomes. It remains to discover how far the specific effects of genes in development are part of the same or a similar system of nucleoprotein metabolism; the rôle of the heterochromatic regions in variegation suggests that such a possibility is not unlikely.<sup>14</sup>

\* Carnegie Institution of Washington; during this work, International Fellow of the Rockefeller Foundation.

<sup>1</sup> Heitz, E., *Zeitschr. f. Zellforsch.*, **20**, 237-287 (1933); Kaufmann, B. P., *J. Morph.*, **56**, 125-165 (1934).

<sup>2</sup> Schultz, J. (unpublished). Kaufman, B. P., *Zeitschr. f. Zellforsch.*, **28**, 1-11 (1938).

<sup>3</sup> Morgan, L. V., *Genetics*, **18**, 250-283 (1933).

<sup>4</sup> Schultz, J., and D. G. Catcheside, *J. Genetics*, **35**, 315-320 (1937).

<sup>5</sup> Rhoades, M. M., *Genetics*, **16**, 375-385 (1931).

<sup>6</sup> Caspersson, T., and Jack Schultz, *Nature*, **142**, 294 (1938).

<sup>7</sup> See Weigert, F., *Optische Methoden der Chemie*, Leipzig, p. 181 (1927).

<sup>8</sup> Heitz, E., *Planta*, **12**, 774-844 (1931); McClintock, B., *Zeitschr. f. Zellforsch.*, **21**, 294-328 (1931).

<sup>9</sup> Schultz, J., quoted in Morgan, Bridges and Schultz, *Yearbook Carnegie Inst. Wash.*, **33**, 274-280 (1934); these PROCEEDINGS, **22**, 27-33 (1936); *Proc. 7th Int. Gen. Cong.* (in press, 1940).

<sup>10</sup> Noudjin, N. I., *Nature*, **137**, 319-320 (1936); *Bull. Biol. Med. Exp. URSS*, **5**, 548-551 (1938); and others.

<sup>11</sup> Lesley, M. M., *Genetics*, **23**, 485-493 (1939).

<sup>12</sup> Bauer, H., *Zool. Jahrb.*, **56**, 239-276 (1936).

<sup>13</sup> Hammarsten, E., *Biochem. Zeitschr.*, **144**, 383 (1924); see also Frey-Wyssling, A., "Submikroskopische Morphologie des Protoplasmas und seiner Derivate," *Protoplasma Monographien*, **15**, 317 (1938).

<sup>14</sup> The data reported in this and the preceding communication were presented in part in papers read at the Seventh International Genetics Congress (1939). The work has been supported by funds from the Rockefeller Foundation and from the Stiftelsen Thérèse och Johan Anderssons Minne.

*ARE DIFFERENCES IN SIZE BETWEEN PARTS OF THE BODY  
DUE TO GENERAL OR SPECIFIC FACTORS?*

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Communicated June 12, 1940

The dispute between Castle<sup>1</sup> and Sumner<sup>2</sup> on the problem expressed in the above title may perhaps be indirectly resolved by results obtained by the writer in a different connection. Castle maintains that the genetic influences determining total size in rabbits are general ones affecting all parts of the skeleton simultaneously, while special factors (if any) limiting the size of particular bones play only a subordinate rôle.<sup>3</sup> Sumner, however, believes that size factors specific to certain limbs and parts of the body are more important than general factors in determining body size as a whole. Davenport holds what appears to be an intermediate view, namely, that general factors control growth "only to a degree that may be estimated as less than a half." Shull supports Castle's contention.

The present writer, like Davenport, has been working on measurements of man, not of rabbits, and has not been primarily concerned with the problem of the *inheritance* of human size.<sup>4</sup> Nevertheless, the observed results obtained by him seem to admit of a genetic interpretation.

Briefly, a large number of anthropometric measurements of several groups of adult persons were obtained. These measurements were then intercorrelated and analyzed by means of the factorial techniques currently used in statistical psychology. The object of the analysis was to extract a minimum number of factors accounting for a maximum amount of the total variance of the traits. The measurements include head length, head breadth, head circumference, head "diagonal," trunk length, chest breadth, chest depth, chest circumference, stature, sitting-height, arm length, leg length, shoulder breadth, pelvic breadth, pelvic circumference, waist circumference and various facial measurements.

The samples consisted of 164 male and 106 female adult psychotics and of 50 normal adult males.

In numerous analyses of the measurements of the subgroups, it was found that the bodily parts could best be regarded as determined by two major growth reactions, one governing magnitude in all physical dimensions and the other governing disproportionate growth in length measurements on the one hand, or in circumferential measurements on the other. In other words, we may consider individual differences in the external measurements of the body to be determined by a process making for general magnitude and a process making for proportions of the parts. The variance of the former is much greater than that of the latter.<sup>5</sup>

In order to get a measure of a person's mass or bulk in the linear sense, corresponding to a ponderal measure or to the measure of the surface area of the body, it is necessary to obtain a weighted mean of representative measurements of the body in all its three dimensions. This can be provided by the first factor saturations in the traits which are measured. Now even when we know a person's bulk, we do not know his shape or proportions. We do not know whether he has relatively long limbs and short trunk or vice versa, we do not know his head shape and so on. This information can be supplied by weighting the initial measurements with saturations of the traits in the second factor.

The amount of variance accounted for by the first and second factors varied in the different subgroups as may be seen below:

	PERCENTAGE OF TOTAL VARIANCE ACCOUNTED FOR:		
	BY FIRST FACTOR	BY SECOND FACTOR	
$N = 64$ males	31.9	12.2	14 traits analyzed
$N = 50$ "	43.6	24.1	14 " "
$N = 62$ females	34.4	12.5	12 " "
$N = 86$ males	42.1	16.8	8 " "
$N = 86$ "	35.0	14.6	6 " "
$N = 62$ females	37.2	14.1	8 " "
$N = 33$ "	29.7	9.9	8 " "

We may hazard the hypothesis that there are genetic influences determining growth or size of the body in all its dimensions, vertical, horizontal and sagittal. Over and above this tendency to grow to a certain bulk or magnitude, we may postulate the existence of genetic factors making for excess or differential growth longitudinally on the one hand, or circumferentially on the other.

If this hypothesis is accepted, the views of Castle, Sumner and Davenport must all be modified. General factors, in our analysis, appear to account for between twice and three times the amount of variance as specific factors. Davenport and Sumner would both seem to be underestimating the significance of general factors while Castle seems to overestimate them.

The view to which we are led here conforms well with that put forward by Pearl in his discussion of individual differences in bodily habitus. The following statement of Pearl sums up the position well: "The vertebrate plan of structure comprises a principal and primary bodily axis which is the longitudinal one, with cephalic and caudal ends. The secondary axes are two in number and at right angles to each other, the dorso-ventral axis and the lateral axis. The external form—somatology or bodily habitus—of such an animal is plainly bound in general to depend upon the relative or proportional growth along each of these axes."<sup>6</sup> I would like to add that growth in bulk proceeds in all three dimensions whereas growth in shape or

proportions proceeds differentially in the first *or* in the second and third dimensions.

<sup>1</sup> Castle, W. E., *Proc. Nat. Acad. Sci.*, 10, 19-22 and 181-182 (1924).

<sup>2</sup> Sumner, F. B., *Ibid.*, 10, 178-180 (1924).

<sup>3</sup> Castle, W. E., *Carnegie Inst. Publ.*, No. 320 (1922).

<sup>4</sup> Cohen, J. I., *Nature*, 144 944 (1939).

<sup>5</sup> Cohen, J. I., *J. Mental Science*, 86, No. 362 (1940).

<sup>6</sup> Pearl, R., *Constitution and Health*, London, Kegan Paul (1933).

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## A PENTAPLOID LARVA OF THE NEWT, *TRITURUS VIRIDESCENS*

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Communicated July 11, 1940

During the past two years a search has been made for exceptional polyploid individuals among salamander larvae, by means of chromosome counts in amputated tailtips. Several triploid larvae have been found in the common newt, *Triturus viridescens*,<sup>1</sup> and triploid as well as tetraploid larvae in the two-lined salamander, *Eurycea bislineata*.<sup>2</sup>

In November and December, 1939, about forty larvae of *Triturus viridescens* were raised by the students in a course on animal development, from eggs which had been obtained by pituitary implants. On December 15 the larvae were turned over to Miss Rita Crotta for fixation. While examining the animals she noticed a suspicious pigment pattern in one larva which had larger but fewer melanophores than the rest. This type of pigment pattern had been found before to be a reliable indication of polyploidy.<sup>3</sup> Moreover, the pattern seemed to be sufficiently different from that previously observed in triploid larvae to indicate a higher degree of polyploidy, probably tetraploidy.

The tailtip of this larva was at once amputated, fixed and stained *in toto*. The nuclei of the various tissues were much larger than in the diploid or triploid tailtips. Unfortunately, the epidermis of the tailfin, in which the chromosomes can be most easily counted, contained only very few mitotic figures, and none of these were in metaphase.

Within two weeks the larva had regenerated a small new tailtip. This was amputated together with a piece of the original tail. This second tailtip preparation contained one good metaphase in the regenerated portion. In this large and crowded figure fifty-four chromosomes could be counted, a number which is as close to the pentaploid, fifty-five, as one could expect considering the difficulties of the enumeration.

A week later the whole animal was fixed, and the posterior end of the tail, with a small regenerated cone, was stained and mounted. In this third tailtip preparation one fairly good metaphase was discovered which contained certainly more than forty-eight chromosomes.

While both counts indicated that the larva was more than tetraploid, probably pentaploid, the evidence was not considered sufficient. An extensive series of measurements of nuclei was planned with the expectation that a comparison of the size of the nuclei with that in diploid and triploid larvae would contribute reliable evidence of the chromosome number. However, when the whole animal was sectioned later on, a considerable number of mitoses were found in the central nervous system and the intestine. In the former, the figures are too small and crowded to allow even an approximate count. In the intestine, the cells are larger, and the chromosomes more widely spaced. Still there is too much overlapping of the chromosomes in intact metaphase plates which are complete within a single section and normally considered to be the most reliable figures for chromosome counts. However, several metaphase plates had been divided by the knife in such a way that few chromosomes were broken, and a count of the intact elements in the two adjacent sections was possible. Since all the chromosomes of *Triturus viridescens* are V- or J-shaped, fragments of chromosomes not containing the spindle attachment point are quite easily recognized. In six metaphases from various regions of the intestine the following chromosome numbers were counted:

54 (very few fragments present),  
53 ( " " " " ),  
at least 52,  
" " 51,  
" " 50,  
" " 50.

These numbers include only those chromosomes that could be made out clearly. Moreover, one or more chromosomes may have been missed if they were cut at or near the spindle attachment point and were therefore recorded as fragments. It is thus highly probable that the actual number in all these figures is pentaploid, fifty-five.

A high degree of polyploidy is also indicated by the very large size of the individual nuclei in the various tissues of the tailtip (Figs. 2 to 7). For instance, the nuclei of the epidermis cells, which are flat discs, appear more than twice as large as those of the diploid larva (Fig. 2). When twenty nuclei of each animal were drawn on cardboard and weighed, the ratio of the weights was found to be close to five to two. The cell boundaries are not clearly visible in tailtip preparations except in the gland cells in the epidermis (Fig. 7) which show that the cell size has increased in proportion



to the size of the nucleus. This is also demonstrated by the increase in size of the melanophores over the whole body of the animal (Figs. 8 and 9).

Since rather complete records of the development of all eggs had been kept by the students in the course, it was possible to trace the development of the pentaploid back to the gastrula stage. As far as the records show, the early development of the pentaploid embryo proceeded normally and at the same rate as that of the diploid embryos. When the pentaploid came under closer observation as a young larva, at the age of three weeks, it did not differ markedly from the diploid controls, with the exception of the pigment pattern. A photograph taken a few days later shows that the pentaploid was only slightly larger than the control (Fig. 8). The absence of pronounced gigantism agrees well with the observations on triploid and tetraploid salamander larvae reported previously.<sup>1, 2, 3</sup> At all levels of

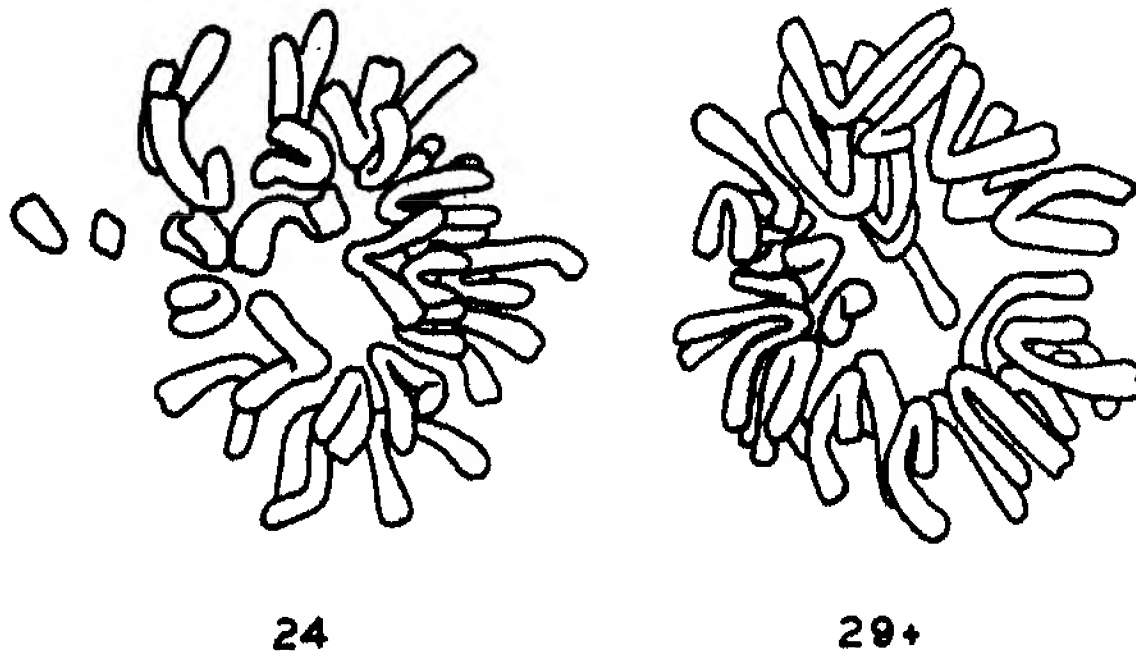


FIGURE 1

Metaphase plate from intestine containing at least 53 chromosomes in two sections. Camera lucida drawing.  $\times 1533$ .

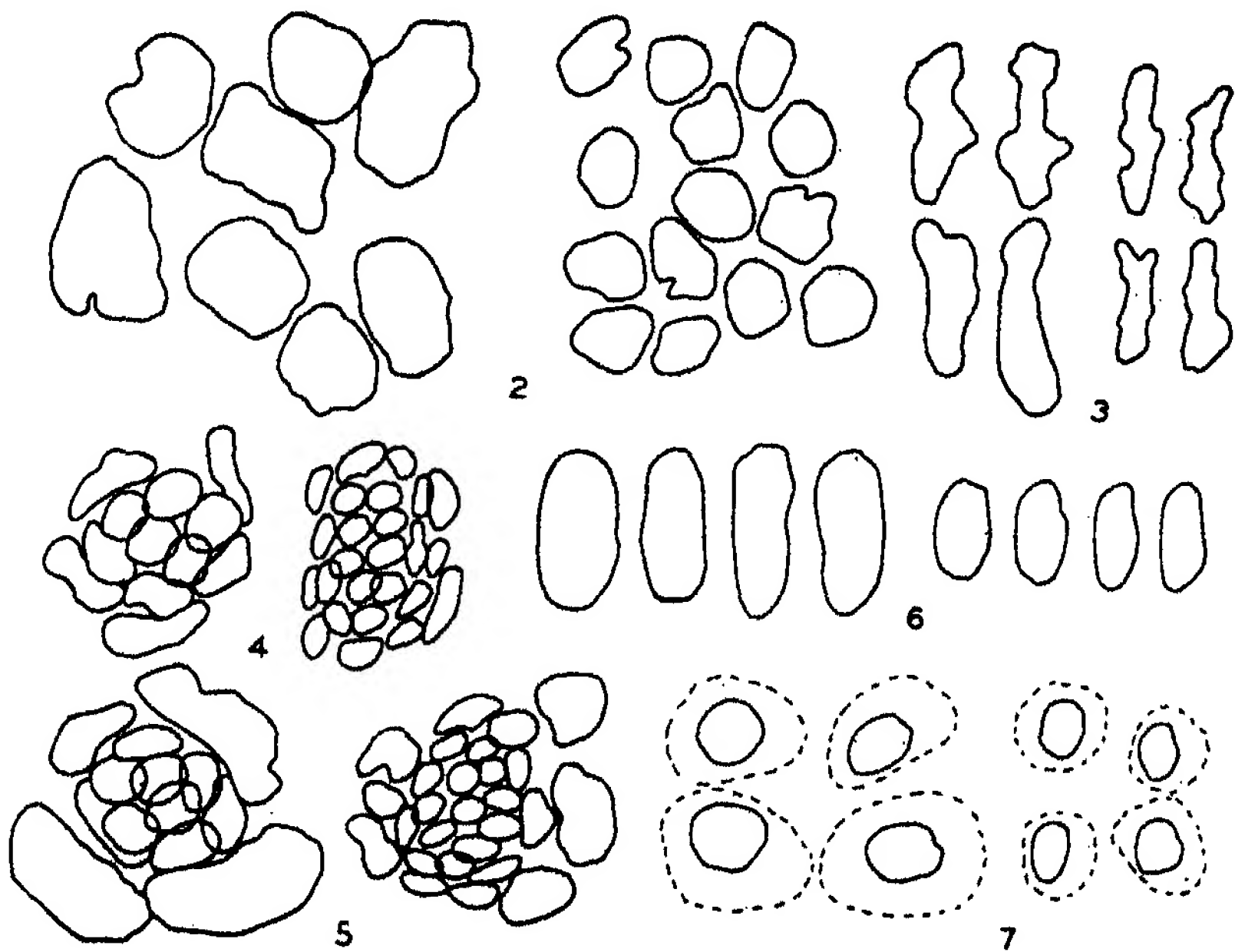
polyploidy, the increase in cell size is compensated by a corresponding decrease in cell number. This is well shown by a comparison of pentaploid and diploid lateral line organs in the tailtip preparations (Figs. 4 and 5).

The pentaploid began to feed on small *Daphnia* and pieces of *Enchytraeus* at the age of three and a half weeks, at the same time as the controls. The digestion of pieces of *Enchytraeus* seemed to proceed somewhat more slowly; it was observed twice that, twenty-four hours after a meal, some of the food was still in the stomach while in the controls it had all passed into the intestine. The rate of heart beat was normal; at the age of five weeks it was 76 per minute, as compared with 76 and 78 in two diploid larvae. The color of the heart, however, was light pink instead of deep red as in the controls, because of the smaller number and larger size of the erythrocytes. The difference in size and number of the red blood cells passing through the heart and gills was clearly visible under the binocular.



On several occasions it was noticed that the pentaploid reacted more slowly than the diploid larvae. Furthermore, from the age of four weeks on, fluid began to accumulate in the body cavity. The ascites gradually became more severe and extended to the heart region (Fig. 9).

On January 3, 1940, the pentaploid and a control were photographed and



FIGURES 2 TO 7

Camera lucida drawings of nuclei from different tissues of tailtip preparations. In each figure the pentaploid nuclei are on the left.

2. Epidermis of tailfin.  $\times 270$ .
3. Connective tissue of tailfin.  $\times 614$ .
4. Nuclei of corresponding lateral line organs on axis of original tailtip (No. 4 from end).  $\times 313$ .
5. Nuclei of corresponding lateral line organs from third tailtip (last organ on dorsal fin).  $\times 313$ .
6. Nuclei of erythrocytes, from capillaries of tailfin.  $\times 614$ .
7. Nuclei and cell boundaries (broken lines) of gland cells (Leydig cells) in tailfin.  $\times 270$ .

stayed in a solution of chloretone 1:5000 for over thirty minutes, a treatment which had never been found to have any harmful effect on diploid or triploid larvae. On the next morning the control was completely normal while the gills of the pentaploid showed the effects of an overdose of chloretone, i.e., an almost complete reduction of the small branches and a strong

curvature of the main stems. Because of its poor general condition the animal was preserved on the following day.

While the morphological effects of pentaploidy in this larva were thus rather slight, the high chromosome number seemed to have more serious physiological consequences, as is indicated by the early appearance of ascites, the reduced reactivity and the increased susceptibility to chloretone.

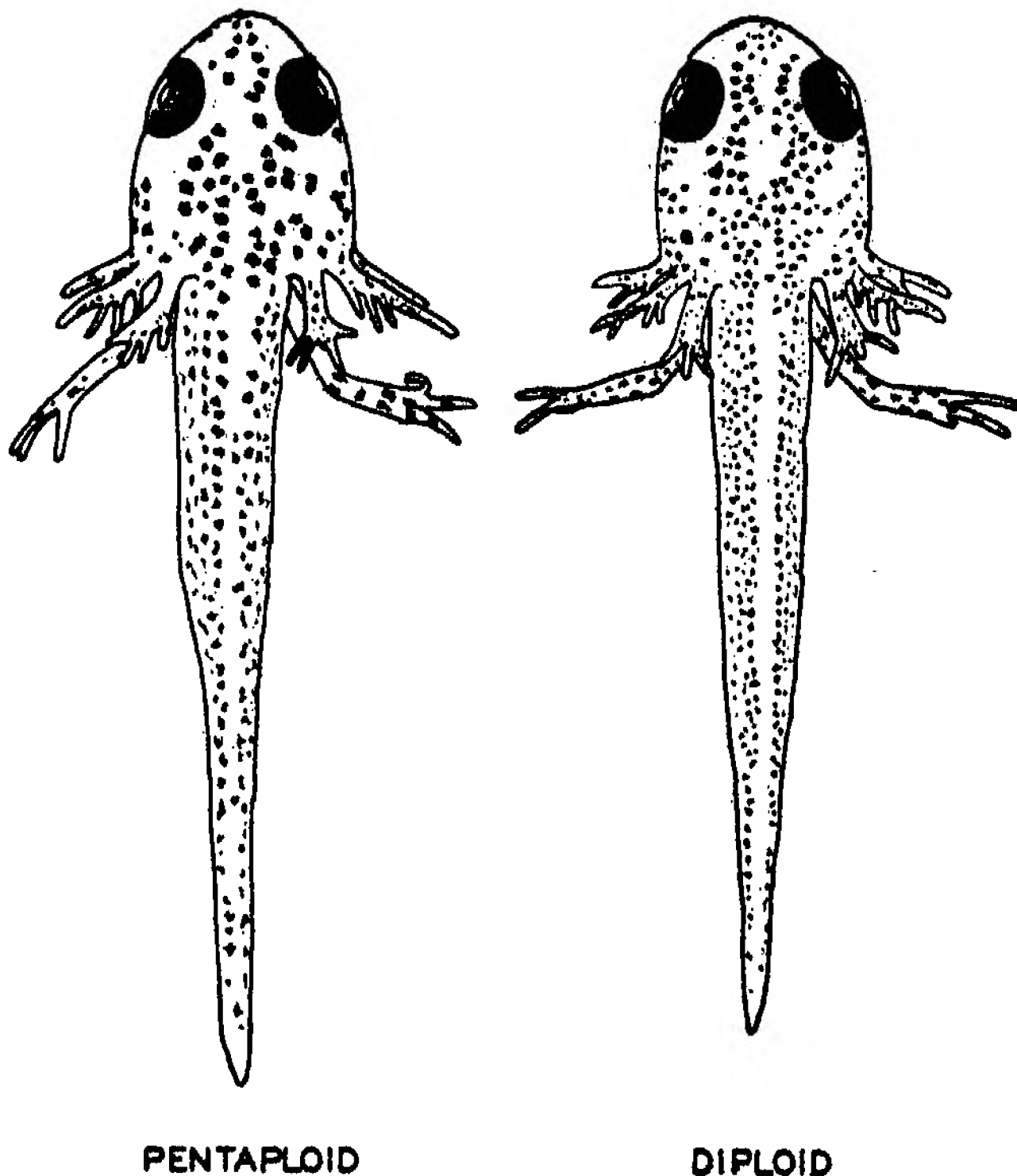


FIGURE 8

Pentaploid larva and diploid control,  $3\frac{1}{2}$  weeks old. The pentaploid appears normal except for pigment pattern and slightly larger size. In both larvae the melanophores on head and body are "contracted." Tracings of photomicrographs.  $\times 10$ .

It is possible that these phenomena were all connected with the great increase in cell size and the reduction in cell number in the various organs and tissues. The microscopical anatomy of the larva is under investigation at present and will be described elsewhere.

It is significant that the viability of the pentaploid salamander larva was much lower than that of the two tetraploid larvae of *Eurycea bislineata*

which were raised in 1939; these in turn were not as vigorous as the triploid larvae of both *Eurycea* and *Triturus viridescens*. Unfortunately, tetraploid larvae have not yet been found in *Triturus*, and attempts to induce tetraploidy by cold treatment of unsegmented eggs during the first cleavage mitosis have not been successful so far (unpublished observations of M. Perrot). There exists, therefore, still a gap in the polyploid series in this species which now extends from haploidy<sup>4, 5</sup> to pentaploidy.

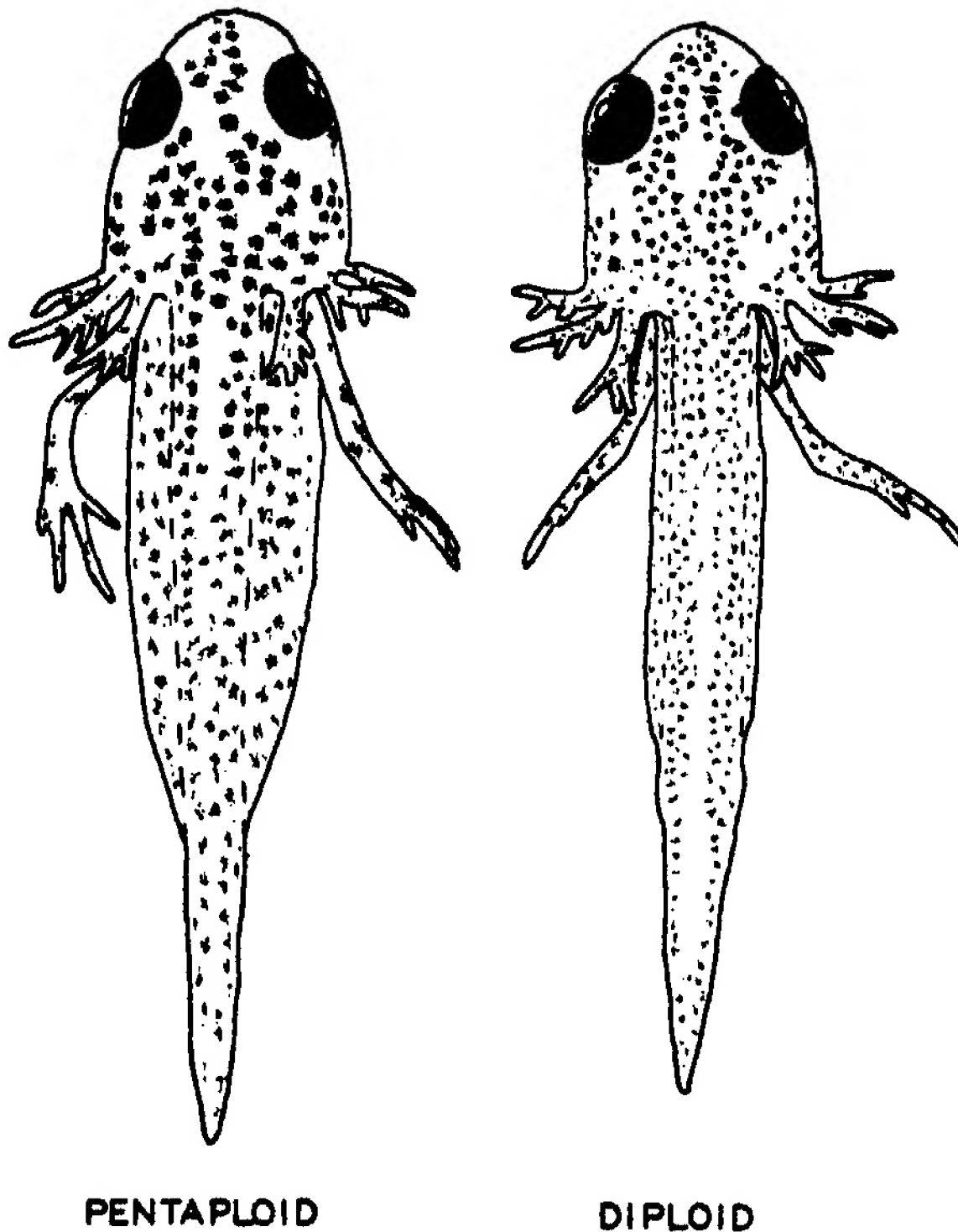


FIGURE 9

The same larvae as in figure 8, 5½ weeks old. Body cavity of pentaploid distended with fluid (ascites). In both larvae the melanophores are "expanded." Tracings of photomicrographs.  $\times 10$ .

No definite information is available concerning the origin of this exceptional pentaploid embryo. Triploid salamander larvae presumably arise in nature from the union of an exceptional diploid gamete with a normal haploid gamete. Tetraploid individuals would originate if two exceptional gametes should meet, or if a doubling of the chromosome number should take place during the first cleavage mitosis. Furthermore, additional sets

of chromosomes might be supplied by supernumerary spermatozoa, since the eggs of newts and other salamanders are normally polyspermic. Usually, the accessory sperm nuclei remain well separated from the pronuclei and degenerate before the first cleavage.<sup>6</sup> On rare occasions, however, two spermatozoa may possibly penetrate into the egg so near each other that the nuclei fuse, as is frequently seen in highly polyspermic eggs which do not cleave. In eggs of the salamander, *Hynobius retardatus*, which seem to be monospermic as a rule, Makino<sup>7</sup> has actually observed the fusion of three nuclei. However, whether such a situation would lead to a bipolar triploid mitosis or to a tetrapolar mitosis with irregular distribution of the chromosomes, as it does in dispermic sea urchin eggs, is not known, since the behavior of the division centers associated with the sperm nuclei has not been observed.

<sup>1</sup> Fankhauser, G., *Proc. Am. Phil. Soc.*, 79, 715-739 (1938).

<sup>2</sup> Fankhauser, G., *Jour. Hered.*, 30, 379-388 (1939).

<sup>3</sup> Fankhauser, G., *Jour. Morph.* (in press) (1940).

<sup>4</sup> Kaylor, C. T., *Jour. Exp. Zool.*, 76, 375-394 (1937).

<sup>5</sup> Fankhauser, G., and R. B. Griffiths, *Proc. Nat. Acad. Sci.*, 25, 233-238 (1939).

<sup>6</sup> Fankhauser, G., *Jour. Exp. Zool.*, 62, 185-225 (1932).

<sup>7</sup> Makino S., *Jour. Fac. Sci., Hokkaido Imp. Univ., Ser. VI, Zool.*, 3, 117-167 (1934).

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## UNEQUAL BREAKS IN TWO SISTER CHROMATIDS INDUCED BY X-RAYS IN *DROSOPHILA MELANOGASTER*

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A great deal of work, both experimental and observational, has been done on the number of strands per chromosome at different stages of the nuclear cycle, the time of splitting of the chromonemata and the effects of irradiation on split chromosomes.

From genetical experiments we have the results of Patterson<sup>1</sup> on mosaics derived from irradiating sperm of *Drosophila*, from which he concluded that the chromosomes were already split in some of the sperms (about one in seven) at the time of irradiation, but not in others. Moore,<sup>2</sup> who obtained similar results, supposed that the chromosomes of all the sperm were split at the time of breakage; mosaics would result from single chromonema breaks, but both chromonemata could be broken simultaneously at the same level, and sperm thus affected would give rise to flies with homogeneous mutant tissues.

Moore's conclusions are in keeping with the cytological data of Nebel<sup>3</sup>

and Sax.<sup>4</sup> These workers have shown by irradiation experiments on *Tradescantia* that a single x-ray "hit" can break either one or two chromonemata at a given point of a split chromosome.

Kaufmann<sup>5</sup> and Kaufmann and Bate<sup>6</sup> have published data on duplications in *Drosophila* salivary chromosomes. These studies show that one of two sister chromatids may be broken at one point, while both chromatids are broken at a second point in the same chromosome. In this paper a further example of differential breakage of two sister chromonemata will be described.

*Description and Results.*—The progenitor of N264-86 mutant is a single notch female found among the offspring of  $\gamma$  *pn* females which were mated with x-rayed wild-type Swedish *B* males. Genetic tests showed that this notch is a dominant sex-linked character, lethal when homozygous and hemizygous, and that it belongs to the Notch series of changes localized in the 3.0 region of the X-chromosome.

The N264-86 females heterozygous for diminutive (*dm*) had diminutive bristles, and some of them showed mosaic spots covered with long, wild-type bristles. Since mottling is a characteristic feature of chromosomal rearrangements involving heterochromatin, this behavior of *dm* suggested that such rearrangement might have occurred in N264-86. Cytological studies confirmed this indication. They showed that a segment of the chromosome was taken out of the Notch region and inserted into heterochromatin of 4 at 101F.

Since the fourth chromosome segregates freely from the X-chromosome, the inserted piece also segregates independently of the X-chromosome from which it came. Consequently two recombination classes are found: (a) hypoploid class with the deficient X-chromosome but without the fourth carrying the insertion, and (b) hyperploid class with the insertion and the normal X-chromosome. The hypoploid class is viable and fertile in heterozygous females, and the hyperploid class is viable and fertile in both sexes.

*Analysis of Deficiency.*—Cytological analysis indicates that this deficiency involves the region from 3C8 to 3E5, inclusive (Fig. 1). In this study salivary glands were used from larvae which had one deficient and one normal X-chromosome. In a great majority of figures obtained with that material the bands present in both chromosomes show close synapsis. In order to confirm the analysis on unsynapsed chromosomes, slides were prepared from the salivary glands of larvae which had one deficient chromosome and the other carrying the translocation (1.4) 258-18, in which the tip of the X-chromosome following the band 3C4 is exchanged with 4. In many figures of that material X-chromosomes are unsynapsed at the distal end.

Genetic tests showed that the deficient chromosome carries the wild-

type alleles of *w* and *ec*, and that it is either deficient or carries recessive alleles of *rst*, *fa* and *dm* (Fig. 1).

*Analysis of the Inserted Segment.*—Cytological study shows that a segment from 3C7 to 3E5 is inserted into heterochromatin of 4 at 101F, in its normal position in relation to the centromere. By genetic tests it was determined that the loci *fa* and *dm* present in the segment show mottling (Fig. 1).

*Discussion.*—All adequately tested Notches behave genetically as deficiencies for *fa* and *spl*, and some in addition behave as deficiencies for the adjacent loci such as *w* and *dm*. Cytological studies of various Notches indicate that some do not show any cytologically detectable deficiency, while others are deficiencies for smaller or larger segments centering in the 3C region of the Bridges' salivary chromosome map. Since all cytological deficiencies include band number 3C7, that band is considered as representing the Notch locus. Similar evidence indicates that 3C1 represents the *w* locus, 3C4 *rst*, 3D1.2*dm* and 3F 1.2 *ec*.

Both cytological and genetic evidence indicates that the inserted segment carries the locus *fa*, since the 3C7 band has been located in the insertion and since hyperploid flies show mottling in the *fa* locus. Cytological observations indicate that the *fa* locus is not involved in the deficiency. Genetic evidence in support of this assumption is impossible to obtain since a change in the *fa* locus has occurred which behaves genetically as a deficiency. Such changes are not rare in the neighborhood of a breakage point. However, in this case the *fa* locus is not the only one which has been changed, but also the *rst* locus which is represented by the fourth band from the break. In this instance a short region involving not less than four and not more than six bands is affected. We have studied 27 breaks in the same region and this is the only case where reattachment to euchromatin produced a block effect. That suggests that such occurrences are not frequent.

Cytological evidence shows that the inserted piece is one band longer than the deficiency. If it were the reverse the interpretation would have been simple, because the total number of all bands in the rearrangement would have been one less than the total number of bands in the original chromosome, and the loss of one band could easily have been accounted for. However, the total number of bands in the rearrangement is one in excess of the total number in the treated chromosome. This increase in the number of bands could occur if the chromosome were split at the time of the break and if two chromatids were broken at different places. In subsequent rearrangements the longer of the two segments and the chromatid with the shorter deficiency were recovered.

A picture of this situation can be obtained from the diagram in figure 1. On the right side the limits of both the deficiency and the inserted segment



coincide. At the time of breakage the chromosome might not have been split at this point, or if it were split the breaks in both chromonemata must have coincided. However, on the left side the inserted segment is longer than the deficiency and the chromosome must have been split in this region at the time of the break and the breakage points in the two chromonemata did not coincide but were one band apart.

Now, what is the probable mechanism which produced these changes? It is known that the rearrangement involves chromosomes which are brought into the egg by the x-rayed sperm. Therefore the breaks were undoubtedly caused by x-ray treatment. The right break, which occurred

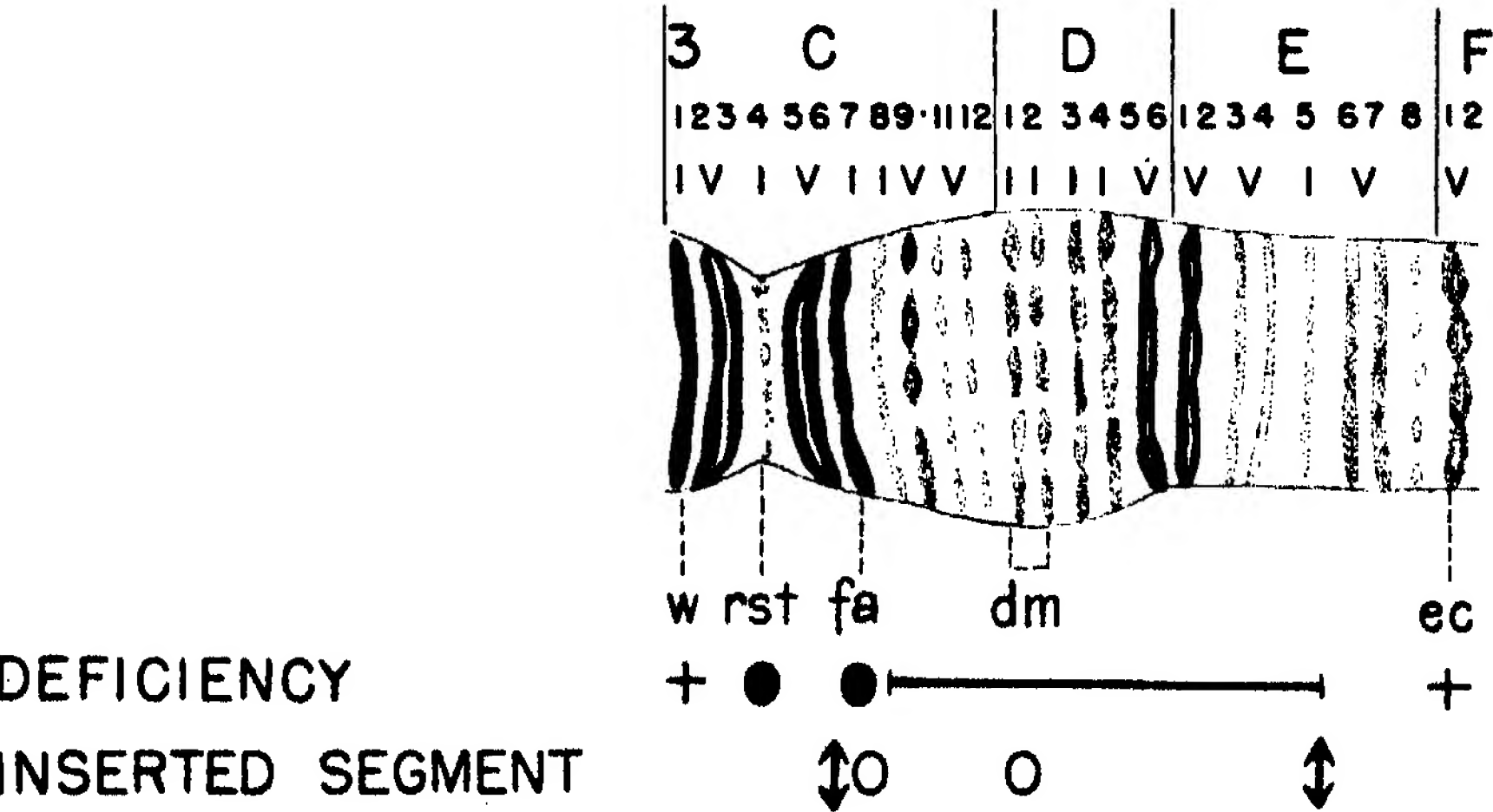


FIGURE 1

Above, section of normal salivary chromosome. Below, changes in the two affected strands: straight line shows extent of deficiency, + = unaffected gene, full O = gene showing mutation; in the other strand, arrows show position of breaks, O = gene producing mottled effect.

at the same point in both of the recovered strands, might have been caused (a) by a single electron hit causing a break in the chromonema before it was split, (b) by a single electron hit causing breaks at the same point in two strands, or (c) by two independent hits each causing a break in one of the two strands at approximately the same point. The left breaks, which are at different points in the two recovered chromatids, might have been caused by (a) two independent hits, each producing a break in one of the two chromatids of the chromonema, or (b) one hit producing two breaks, one in each chromatid, at two different points of the chromonema.

Genetic analysis of the region adjacent to the left break gives the clue for deciding between two possibilities. It is evident from figure 1 that in

the strand involving a deficiency the loci *fa* and *rst* are affected, namely, that a section of the chromosome adjacent to the break and extending through at least four bands has been changed. It is known that a break in the chromosome sometimes induces a change in the locus adjacent to that break. We have a few cases where a locus a short distance from the break has been affected, presumably through the position effect. However, such a condition is very rare and seems to be specific to the locus. Except for the present case it has never been observed for the *rst* locus. The incidence of breaks induced in the two strands at different places but close together is not known, but since this is the first case of this type on record it is justifiable to assume that it must be low. Since the frequency of each of these two events is low it seems very probable that their occurrence together indicates that they were induced by the same cause, which in the present case means by a single electron hit. This could be brought about through the action of a single excitation shock given by an electron to the surrounding matter and diffused over a relatively large area covering two strands in width and four bands or about 1.5 microns in length. However, this effect took place in the sperm where the chromosomes are very likely coiled and thus the length of the affected region may be considerably smaller than in the salivary gland chromosomes where the chromonema is stretched. It is reasonable to assume that salivary gland chromosomes are about one hundred times as long as chromosomes in the sperm, thus a 1.5 micron section of salivary chromosomes is condensed to about 150 Å in the sperm. The spread of the physical excitation phenomena over such distance is not contrary to physical expectation.

*Summary.*—Cytogenetic analysis of N264-86, which was obtained from an x-rayed male, shows that two strands of the X-chromosome were affected by the treatment. One of the strands has a deficiency from 3C8 to 3E5, inclusive, and in addition changes in *fa* and *rst* loci, while a piece from 3C7 to 3E5, inclusive, has been taken out from the other strand and inserted into chromocenter of 4. Thus the right breaks occurred at the same point, while the left breaks occurred at different points but close to each other. It seems probable that the left breaks and the changes in adjacent loci were induced by the excitation produced by a single electron hit.

<sup>1</sup> Patterson, J. T., *Genetics*, 18, 32-52 (1938).

<sup>2</sup> Moore, W. G., *Genetics*, 19, 209-222 (1934).

<sup>3</sup> Nebel, B. R., *Amer. Jour. Bot.*, 24, 365-372 (1937).

<sup>4</sup> Sax, K., *Genetics*, 23, 494-516 (1938).

<sup>5</sup> Kaufmann, B. P., *Jour. Hered.*, 30, 179-190 (1939).

<sup>6</sup> Kaufmann, B. P., and Bate, R. C., *Proc. Nat. Acad. Sci.*, 24, 368-371 (1938).

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*A NOTE ON THE VARIATION OF DELTA SCUTI*

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The star  $\delta$  Scuti,  $18^h36^m.8$ ,  $-9^\circ9'$  (1900), was found by Fath,<sup>1,2</sup> from photoelectric measures made during July, 1935, and July and August, 1936, to be variable with a range of the order of  $0^m.2$  and a period of  $0^d.19377$ . The radial velocity had already been found by Colacevich<sup>3</sup> to be variable in that period. Neither the light curve nor the radial-velocity curve, however, is regularly periodic, and both curves appear to be in a state of continual change. From the several nights on which simultaneous light and radial-velocity measures were made, it was seen that changes in the light curve are correlated with changes in the radial-velocity curve; and, finally, Fath found that the range of the variation in light appeared to be periodic in a period of  $5^d.24774$ . He suggested that this might be an interference period, between the fundamental period of  $0^d.19377$  and a slightly longer "secondary" period of about  $0^d.20120$ . Such a pair of periods would get into step with each other once every  $5.24774$  days, producing very nearly the observed variation in the range.

It was pointed out by Sterne<sup>4</sup> that the preceding periods could not reasonably be reconciled with the pulsation theory. For any single gaseous star, there are a set of dynamically possible modes of radial oscillation, which differ among themselves in the disposition and number of their nodal surfaces, and in their periods. In the mode of lowest order (mode zero), the displacement vanishes only at the center; in the next mode the displacement vanishes at the center and also over a spherical surface inside the star. The ratio of the period of mode one to that of mode zero depends upon the law of distribution of matter within the star, as well as upon the effective ratio of specific heats,  $\gamma$ , of the mixture of matter and radiation within the star. The ratio of periods was computed for a polytrope of index three by Edgar,<sup>5</sup> for certain particular values of  $\gamma$ . The ratio of the period of mode two to that of mode zero was computed for this same model by Miss Kluyver,<sup>6</sup> who adopted a value of  $20/13$  for  $\gamma$ ; while Sterne,<sup>7</sup> in order

to determine what effect varying degrees of concentration of density would have upon the ratios of the periods, found the periods of all the modes of three other stellar models for all values of  $\gamma$ . The general theoretical result is that no reasonable distribution of density can yield a ratio, between the periods of the zero'th and first modes, that lies as close to unity as the ratio of  $0^d.20120$  and  $0^d.19377$ . The order of the modes that could yield so close a ratio would have to be about the twenty-sixth or higher, and it is nearly impossible to imagine how a limited number of modes of such high order could be maintained in oscillation in a star, with the effective exclusion of modes of lower order.

To see whether it could not be reconciled with the pulsation theory, Sterne<sup>4</sup> rediscussed the observational material. The residuals of Fath's observations from the mean light curve, formed according to the period  $0^d.19377$ , were found not to be periodic in a period  $0^d.20120$  and thus this value of the secondary period was ruled out. The residuals, however, could be fitted by a secondary period of  $0^d.18688$ , or alternatively of  $0^d.15739$ . A third possible value,  $0^d.13603$ , was not in as good agreement with the observations as either of the two longer values. The first of the three observationally plausible values was inconsistent with the pulsation theory; the last two were not obviously inconsistent with it. Fath's observations were made at the Lick Observatory, between about 0.7 and 0.9 of the Julian day, and did not enable one to discriminate between the two most observationally probable values of the secondary period. The two alternative periods fitted the observed magnitudes and the observed ranges equally well, and both fitted an observed sine-term in the ephemeris of the times of median increasing magnitude. Both values of the secondary period, moreover, seemed to fit the radial-velocity measures published by Colacevich.<sup>5</sup> The principal indeterminacy, which arose from the distribution of the observations in time during the hours of darkness at a single observing station, could be removed only by observations made in different longitudes. There was another, and trivial, indeterminacy arising from the seasonal distribution of the observations.

As the result of an arrangement made by Fath through correspondence with the director,  $\delta$  Scuti was observed photoelectrically<sup>6</sup> by the Russian Abastumani Observatory, on Mt. Kanobili in Georgia, during the summer of 1938 while Fath observed the star at the Lick Observatory. Fath has recently discussed<sup>9</sup> the important Mt. Kanobili observations, along with his own. The shorter, of Sterne's pair of suggested values of the secondary period, appears to fit the combined Mt. Kanobili and Lick observations better than the longer value. The value adopted for the secondary period by Fath is  $0^d.157382$ . For the fundamental period he finds  $0^d.193770$ , and he finds evidence also for a third period,  $0^d.095156$ . The corresponding ranges are  $0^m.033$ ,  $0^m.167$  and  $0^m.011$ . Besides these three periods, that

persist throughout the three observational series, he finds small variations that do not persist throughout them all, and still others that appear to be erratic and hardly distinguishable from observational errors.

It is the purpose of this note to indicate the bearing of Fath's recent results on the pulsation hypothesis. The three observed periods are in the ratio of 1:0.812:0.491. As has been mentioned, Miss Kluyver<sup>6</sup> has computed the ratio of the period of the second radial mode, of a polytrope of index three, to that of the zero'th mode. The value of  $\gamma$  that she adopted was 1.54, and the ratio of periods that she found was 1:0.515. A slightly smaller value of  $\gamma$  would make the agreement with the observed ratio, 1:0.491, exact. Unfortunately, the period of the first mode of vibration of the polytrope does not appear to have been correctly computed by anyone, and a comparison with the observed ratio 1:0.812 is not now possible. The periods computed by Edgar,<sup>5</sup> and included with reservations by Sterne<sup>7</sup> in his tables, appear to be inconsistent with Miss Kluyver's computed results and to be too short.

According to the *Henry Draper Catalogue*, the visual magnitude of  $\delta$  *Scuti* is 4.74 and its spectral class is F0. Its revised spectral class is given as F5 by the Lick observers and as F4s by Mount Wilson. The Mount Wilson absolute visual magnitude is +1.4. From the spectral class and absolute magnitude, the star's radius is found<sup>4</sup> to be about 3.6 times the sun's; and from the spectral class, absolute magnitude, and mass-luminosity-temperature relation the mass is found to be about 2.5 times the sun's. The mean density is thus about 0.076 gm./cm.<sup>3</sup>, and with this mean density a polytrope of index three would have the period 0<sup>d</sup>.19377 for its lowest mode if  $\gamma$  were just about 1.54. However, the observational determination of the density is very rough, and but little stress should be laid on the close agreement between the values of  $\gamma$  inferred on the one hand from the ratios of periods, and on the other from the length of the fundamental period. Moreover, the polytropic model can be but an approximation to the actual distribution of density. Nevertheless, it can at least be stated that there does not now appear to be any insurmountable discrepancy between the periods of  $\delta$  *Scuti* and the pulsation theory. The very small non-persistent variations (of the order of one or two hundredths of a magnitude) reported by Fath do not seem to the present author to be serious obstacles. Such small, irregular variations may not be related to pulsations at all, but may be merely superficial in nature, or due to convection currents. It seems improbable that any luminous star should be absolutely constant in brightness, and in Cepheids one should expect the effects of the important pulsations and of the small erratic disturbances to be additive.

It is greatly to be hoped that the periods of the first and second modes of a polytrope of index three may be computed for several values of  $\gamma$



in order to allow a more complete comparison to be made. It is possible that some information may thus be obtained about the distribution of density, since one ratio, but not two, can be fitted by varying  $\gamma$ .

In view of its smallest damping,\* one would in general expect the mode of longest period to exhibit the greatest amplitude. However, the theoretical problem of the relative amplitudes is complicated by the influence, upon the maintenance of the oscillations, of the manner in which the compression-sensitive sources of energy are distributed throughout the stellar interior. Sources concentrated into regions where there are no changes in temperature and density, during the oscillation of a certain mode, cannot be effective in maintaining that mode in a state of oscillation even if the rate of generation of energy increases rapidly with increasing temperature and density. On the other hand, compression-sensitive sources in regions where there are changes in temperature and density, during the oscillation of a particular mode, can be effective in maintaining that mode in oscillation. It is of some interest in this connection to notice that the ranges of the three periodic variations found by Fath in  $\delta$  *Scuti* are in the ratios of 1:0.197:0.066 magnitudes, in decreasing order of periods.  $\delta$  *Scuti* is a member of the  $\beta$  *Canis Majoris* type of stars. The existence of sine-terms, in the ephemerides of cluster-type variables like *RR Lyrae*, indicates that some of these stars also oscillate in more than one mode simultaneously. Classical Cepheids like  $\delta$  *Cephei*, of still longer period, exhibit great regularity in their light curves. The differences between the numbers of modes that are oscillating in some Cepheids, and in others, doubtless arise from differences of model that may involve differences in their distributions of sources of energy and in their compositions.

*Summary.*—From photoelectric observations made in different longitudes, Fath has found (*Lick Observatory Bulletin*, No. 501, 1940) that the secondary period of the star  $\delta$  *Scuti* is probably 0<sup>d</sup>.157382, and he finds evidence for a persistent third period of 0<sup>d</sup>.095156. It is pointed out that these periods and the fundamental period of 0<sup>d</sup>.193770 can be not unreasonably interpreted as the three longest periods of radial oscillation. For a more complete comparison with the pulsation theory, and to gain knowledge about the distribution of density, it is desirable for the periods of the first and second modes of a polytrope of index three to be computed for each of several values of the effective ratio of specific heats.

<sup>1</sup> Fath, *Lick Obs. Bull.*, 17, 175 (1935).

<sup>2</sup> Fath, *Ibid.*, 18, 77 (1937).

<sup>3</sup> Colacevich, *Ibid.*, 17, 171 (1935).

<sup>4</sup> Sterne, *Ap. J.*, 87, 133 (1938).

<sup>5</sup> Edgar, *M. N.*, 93, 422 (1933).

<sup>6</sup> Kluyver, *B. A. N.*, 7, 313 (1936).

<sup>7</sup> Sterne, *M. N.*, 97, 582 (1937).



\* Nikonov, *Bull. Abastumani Ap. Obs.*, No. 3, 27 (1938).

\* Fath, *Lick Obs. Bull.*, 19, 77 (1940).

\* See, for example, reference 5.

## GALACTIC AND EXTRAGALACTIC STUDIES, VIII. A NEW DETERMINATION OF THE PERIOD-LUMINOSITY CURVE

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1. *Introduction.*—The basic significance of the period-luminosity relation among Cepheid variables in all studies of the distances and dimensions of external galaxies encourages every effort to improve its empirical standardization. The coördinates of the period-luminosity curve most commonly used in recent years are those published in Harvard Monograph No. 2, p. 135 (1930). For the derivation there given, the form of the curve is based on Cepheid variables in the Small Magellanic Cloud and in the globular clusters. The zero point of the curve (which happens to give absolute magnitude zero for the median of average cluster-type variables) is based on the parallaxes and motions of the brighter galactic Cepheids. Recently the zero point has been examined critically by R. E. Wilson, who not only had at his disposal a great deal more observational data on radial velocities and proper motions than were at hand for my original derivation of the curve, but has also included in his discussion the effects of space absorption on the magnitudes and galactic rotation on the motions of galactic Cepheids.<sup>1</sup> Wilson finds no need as yet for altering the adopted zero point, the computed correction being  $0.0 \pm 0.2$  for the cluster-type Cepheids and  $-0.14 \pm 0.2$  for the classical Cepheids.

TABLE 1

NEW PERIODS AND MAGNITUDES FOR THIRTEEN VARIABLES

H. V.	LOG P	$\dot{m}$	A	H. V.	LOG P	$\dot{m}$	A
1408	0.214	16.5	0.95	1943	0.210	16.75:	0.5:
1420	0.294	16.5:	>1.0	1964	0.321	16.45:	1.1:
1655	0.207:	16.75	0.4	2002	0.371	16.2	1.0
1675	0.166	16.8	0.6	2114	0.390	16.4	1.1
1731	0.327	16.5	1.0	2173	0.482	16.2	1.3
1928	0.221	16.45	0.9	3610	0.178:	16.8:	>0.6
1823	0.524	16.3	0.9				

It is now possible to reexamine the form of the period-luminosity curve with the aid of much new material on variable stars in the two Magellanic clouds. Altogether we have available the periods and corrected median

magnitudes of 307 variables in the Small Cloud, and 137 in the Large Cloud. To those already published for the Small Cloud,<sup>2</sup> we can now add the list of faint variables given herewith in table 1. More than half of these thirteen stars have periods of less than two days and are valuable therefore in strengthening the fainter end of the period-luminosity curve. In fact, most of these stars had been suspected, on the basis of the insufficient observational material then at hand, to be cluster-type Cepheids, or possibly variables in the period-interval between one and two days where it had been supposed until recently that periods rarely occur.<sup>3</sup> Miss McKibben derived the periods and median magnitudes for the stars of table 1; she and Miss Dowse have assisted in the calculations for the present paper.

TABLE 2

INTERVAL OF LOG <i>P</i>	MEAN LOG <i>P</i>	MEAN <i>m</i>	NO. OF STARS	WEIGHT
0.0-0.2	0.140	16.71	17	1
0.2-0.4	0.297	16.45	53	3
0.4-0.6	0.501	16.22	61	4
0.6-0.8	0.688	15.88	58	4
0.8-1.0	0.897	15.54	39	2
1.0-1.2	1.108	15.03	36	2
1.2-1.4	1.263	14.82	19	1
1.4-1.6	1.485	14.40	17	1
1.6-1.8	1.662	14.05	2	
1.8-2.0	1.877	12.98	3	
>2.0	2.212	12.85	2	

2. *The Period Luminosity Curve.*—The data for the individual stars are plotted in figure 1, where the vertical ordinates are absolute photographic median magnitudes and the abscissae are logarithms of the periods. Practically without exception the periods are very accurately determined, and the considerable scattering therefore represents observational and natural deviations in magnitude. The plotted crosses in the diagram represent means for intervals of two-tenths in the logarithm; table 2 contains these mean values. The fifth column of the table shows the weights adopted in the solution for the straight line in the diagram, for which the equation is

$$m = 17.04 - 1.74 \log P \quad (1)$$

$$\pm .05 \quad \pm .06$$

Although a straight line adequately represents the period-luminosity relation for the interval from 1.2 to 40 days, there is no *a priori* reason for expecting that the complicated phenomenon of Cepheid variation should exhibit throughout its whole range of occurrence a linear relation between *m* and log *P*. In fact, we know that the curve flattens out for periods shorter

than one day, and the present evidence of figure 1 indicates also a deviation from linearity for the longest periods.

To obtain the absolute photographic magnitude as a function of period, the zero point has been based on Wilson's work on classical Cepheids, mentioned above. From his results we find that corresponding to  $\log P = 0.89$ ,  $\dot{M} = -1.83$ . From (1) we have  $m = 15.49$  for  $\log P = 0.89$ . On the

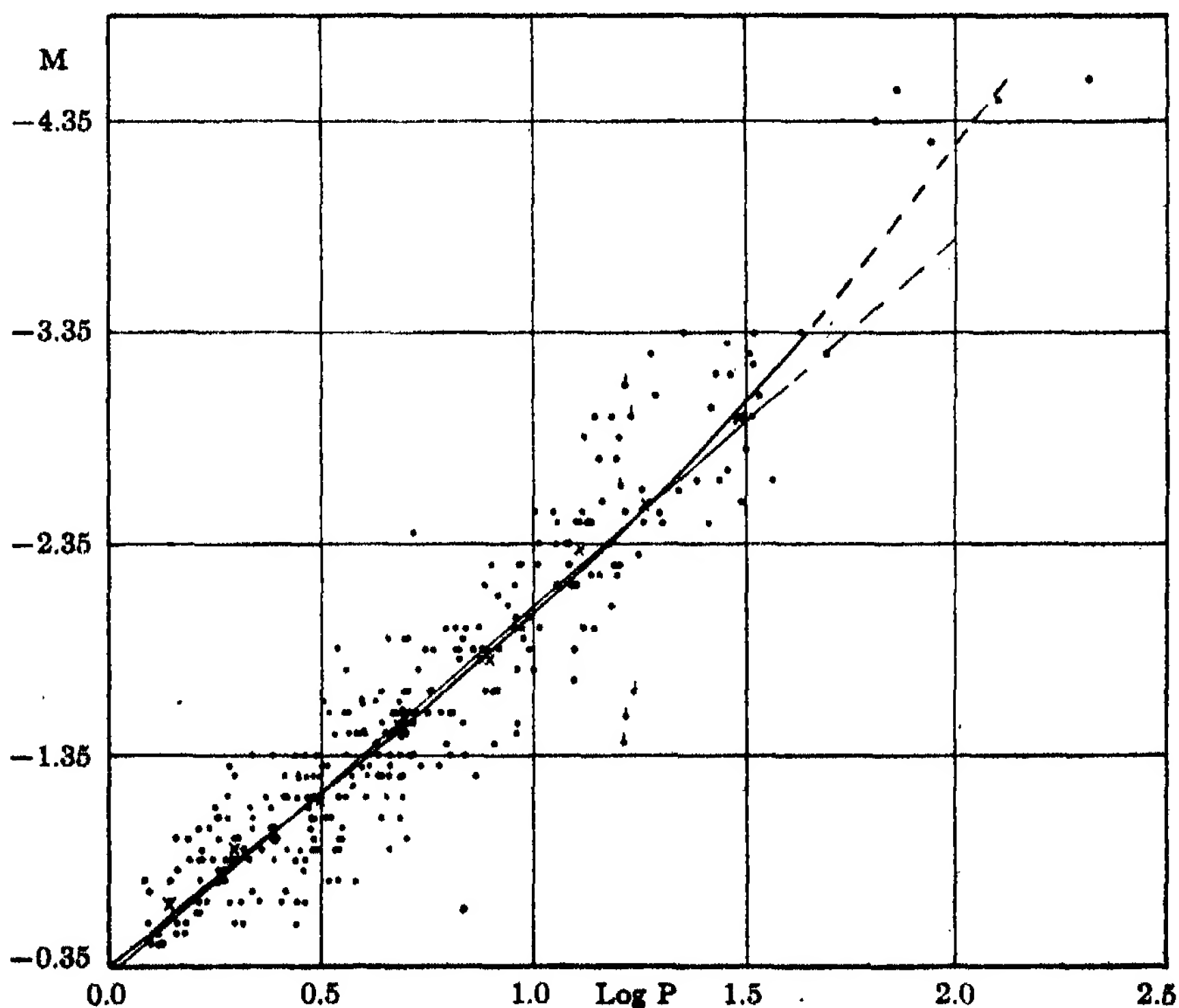


FIGURE 1  
Photographic period-luminosity curve.

absolute scale, therefore, since the modulus is  $m - \dot{M} = 17.32$ , the straight line representation may be written

$$\dot{M} = -0.28 - 1.74 \log P \quad (1')$$

which will be closely approximate for periods between 1.2 and 40 days.

It seems best, however, to represent the observations with the non-linear curve drawn in figure 1, and the coördinates of this curve are those appearing in table 3 as the best values now available for the period-luminosity relation. The last column of table 3 gives the differences between the earlier photographic period-luminosity curve and the present revision.

Not only are there few stars with periods longer than forty days, but their redness and relatively great apparent brightness make them difficult to measure with accuracy or certainty in the magnitude system applicable to the Cepheids of shorter period. The scattered points in figure 1 for  $\log P > 1.6$  may therefore be revised with further study of the magnitude system. A special photometric program is now under way at the Boyden Station of the Harvard Observatory which should stabilize the magnitude sequences for these brighter stars.

In the absence of periods shorter than one day, no revision is offered for the period-luminosity curve fainter than apparent magnitude 17.0, or absolute magnitude  $-0.35$ .

TABLE 3  
COÖRDINATES OF ADOPTED PERIOD-LUMINOSITY CURVE

LOG OF PERIOD	ABS. PG. MAGNITUDE	H. MON. 2 — ADOPTED	LOG OF PERIOD	ABS. PG. MAGNITUDE	H. MON. 2 — ADOPTED
0.1	-0.51	+0.06	1.0	-2.02	+0.13
0.2	0.68	0.07	1.1	2.20	0.13
0.3	0.85	0.08	1.2	2.39	0.13
0.4	1.01	0.08	1.3	2.59	0.12
0.5	1.17	0.10	1.4	2.80	0.12
0.6	1.33	0.11	1.5	3.02	0.09
0.7	1.49	0.12	1.6	3.25	+0.06
0.8	1.66	0.13	1.8	3.73	-0.08
0.9	-1.84	+0.13	2.0	-4.24	-0.36

3. *Concerning Space Absorption in the Cloud.*—The large scatter shown by the individual points in figure 1 is of considerable interest. The material as it stands is fairly homogeneous. The observations have been reduced to the system of a single observer, and the various sequences throughout the Cloud reduced to a common magnitude system.<sup>2</sup> Many factors have contributed to the scatter, but unresolved doubling, thickness of the Cloud in the line of sight, space absorption in the Cloud, Eberhard and background effects and the natural spread of the luminosities for a given period are the most important. A considerable checking of results shows that the spread can be little decreased by more profuse or more precise photometry. The thickness of the Cloud can contribute about two-tenths of a magnitude to the spread. In places, local space absorption appears to play an important rôle; for example, what appears to be the effect of fairly heavy localized absorption is illustrated in figure 2 for six variables with periods almost alike, but with a spread in luminosity of more than 1.5 magnitudes. (In figure 1 these six stars are represented with dots and extruding lines.) The three faintest variables are in the denser part of the Cloud.

It might be expected from figure 2 that the median magnitudes for a given period would be systematically brighter in the outer parts of the sys-

tem because of centralized space absorption. But, after the reduction of all observations to the system of a single observer and a single magnitude sequence, no appreciable general absorption is established. To examine this matter closely, period-luminosity curves were derived separately for the inner and outer regions, and also for the nucleus and for the border regions. The resulting equations below should be compared with (1), and with each other.

$$\text{Inner: } \dot{m} = 17.00 - 1.69 \log P \text{ (143 stars)} \quad (2)$$

$$\pm .06 \quad \pm .07$$

$$\text{Outer: } \dot{m} = 17.06 - 1.79 \log P \text{ (164 stars)} \quad (3)$$

$$\pm .09 \quad \pm .12$$

$$\text{Nuclear: } \dot{m} = 16.84 - 1.44 \log P \text{ (77 stars)} \quad (4)$$

$$\pm .12 \quad \pm .11$$

$$\text{Border: } \dot{m} = 17.01 - 1.70 \log P \text{ (80 stars)} \quad (5)$$

$$\pm .08 \quad \pm .10$$

The mean errors of the parameters indicate the fit of the calculated straight lines to the normal points for intervals of two-tenths of the logarithm of the period. A comparison of the constant terms in the foregoing relations indicates that the absorption in the nucleus and inner regions is not greater than farther out; if anything, the nucleus is the clearest—a somewhat surprising result. But the slope of the period-luminosity curve is also low in the nucleus. Notwithstanding the large mean error, this low value is probably significant, and is indirectly attributable perhaps to the remarkable period-frequency phenomenon discussed in an earlier paper:<sup>3</sup> among the nuclear stars there are relatively few short-period classical Cepheids. If we take a mean slope of 1.57 for the nuclear and border regions, we obtain:

$$\text{Nuclear: } \dot{m} = 16.95 - 1.57 \log P$$

$$\pm .05$$

$$\text{Border: } \dot{m} = 16.94 - 1.57 \log P$$

$$\pm .05$$

An extended study of the variables in nuclear regions is now in progress, with the hope of throwing more light on equation (4), as well as on the frequency of periods and on internal absorption.

Another test of the possible differences in space absorption, when inner and outer regions are compared, is obtained directly from the mean apparent magnitudes of the variables with periods between five and ten days. We have:

	MEAN PERIOD	MEAN MAGNITUDE	NUMBER OF STARS	REDUCED MAGNITUDE
Inner	7.130	15.60	41	15.60
Outer	6.899	15.68	20	15.66
Nuclear	7.126	15.65	27	15.65
Border	6.785	15.75	11	15.72

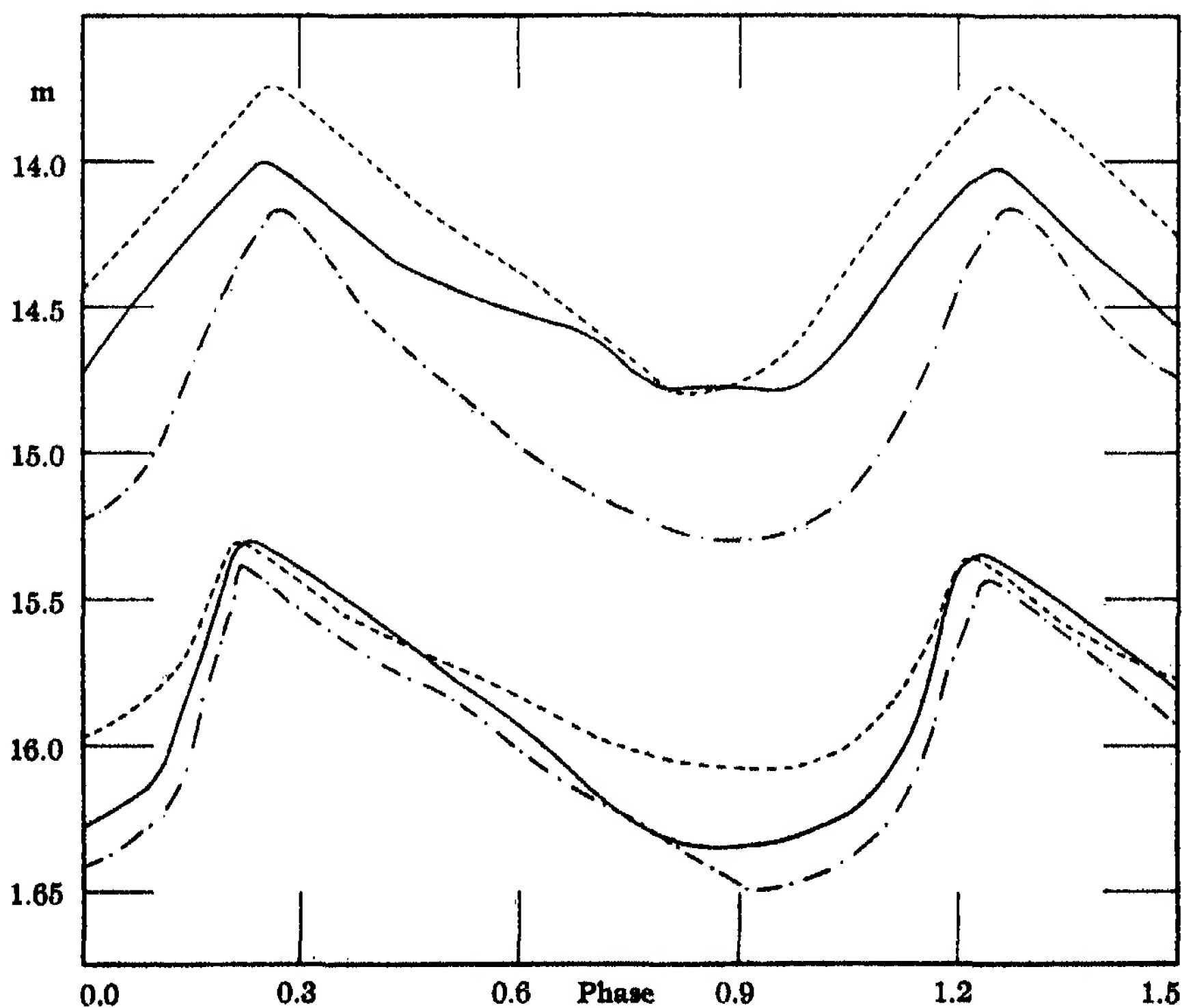


FIGURE 2

Illustration of local space absorption in Small Magellanic Cloud. The variables in descending order (left-hand margin) are H. V. 1954, 1925, 1787, 1478, 1538 and 828. The periods, in days, are respectively, 16.7, 16.8, 16.2, 17.5, 16.4, 16.3. Minimum magnitude for H. V. 1478 is considerably brightened by a partially unresolved companion star. H. V. 1925, also of small range, is not visibly double. The three faintest variables are in the densest part of the nucleus; the three brighter variables are well out of the dense nucleus, but not in border areas.

where the last column contains magnitudes reduced to the period 7.13 days. The result is the same as before, since, in photographic magnitudes,

$$\text{Inner} - \text{Outer} = -0.06 \pm 0.06 \text{ (m. e.)}$$

$$\text{Nuclear} - \text{Border} = -0.07 \pm 0.06 \text{ (m. e.)}$$



and the center of the Cloud is if anything the clearest part, so far as these mean results are an adequate test of absorption.

For the 137 stars for which periods have been determined in the Large Magellanic Cloud<sup>1</sup> the linear relation is

$$\begin{aligned} m &= 17.14 - 2.08 \log P \\ &\pm .08 \quad \pm .09 \end{aligned} \tag{6}$$

The higher slope of the curve may indicate merely that the magnitude system differs from that of the Small Cloud, notwithstanding our earlier attempts to harmonize the magnitude sequences. The new southern photometry should clear up this discrepancy.

4. *Distance of the Small Cloud.*—With the revised period-luminosity relation we are able to make a revised determination of the distance of the Small Magellanic Cloud. The new modulus is

$$m - \dot{M} = 17.35,$$

a value differing inappreciably from 17.32, derived ten years ago. Although the modulus is well determined, with a mean error, probably not exceeding a tenth of a magnitude, the distance of the Cloud is indeterminate over a considerable range because of uncertainties as to the correction necessary to allow for galactic space absorption. The galactic latitude of  $45^\circ$  insures fairly high transparency; and from available long-exposure Bruce photographs we have evidence of a high population of external galaxies in the neighborhood of both Magellanic Clouds.<sup>4</sup> But much further analysis of stellar and nebular distribution in the southern hemisphere will be necessary before the distances to the Clouds can be given with close accuracy. For the present we may compute the distance of the Small Cloud on three assumptions as to the total photographic absorption, suggesting that the middle value be used for the present:

ABSORPTION, <i>m</i>	DISTANCE IN KILOPARSECS
0.0	29.5
0.25	26.3
0.5	23.4

5. *Summary.*—The number of classical Cepheids in the Small Magellanic Cloud for which we know the periods and median magnitudes has been much increased recently, and with the standardization of magnitude sequences throughout the Cloud it has been possible to revise the period-luminosity curve. Recent work by Dr. R. E. Wilson on the zero point of the period-luminosity curve makes the revision especially appropriate at this time.

Throughout the interval from 1.2 to 40 days (which covers the periods of most of the variables known in the Magellanic Clouds), a linear relation

between the logarithm of the period and the median magnitude is fairly satisfactory. A non-linear curve is adopted (table 3) which differs but little from the one now in general use. A study of the period-luminosity relation throughout the Small Cloud reveals no measurable difference depending on the density of star distribution.

The scattering of points about the adopted curve (Fig. 1) is significant, and only a part can be attributed to "Cloud thickness." Possibly space absorption within the Cloud is spotty in distribution and accounts for some of the larger deviations, but there seems to be a real dispersion in absolute luminosity for Cepheids of the same period (Fig. 2). The new value obtained for the distance of the Small Cloud (section 4) is affected by the uncertain information on space absorption in the galactic system.

<sup>1</sup> Mt. Wilson Contr. No. 604 (1939).

<sup>2</sup> Shapley and McKibben, *Harv. Circ.* 439, 1-5 (1940).

<sup>3</sup> See fifth paper of this series, these PROCEEDINGS, 26, 105 (1940); *Harvard Reprint* 192.

<sup>4</sup> Shapley, *Harv. Ann.*, 105, No. 8 (1937).

## THE SPECTRUM OF BD +30°3639\*

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Recent investigations have again directed the attention of astronomers to this remarkable object consisting of a carbon Wolf-Rayet nucleus of type WC8, which is surrounded by a nebula showing strong forbidden lines of ionized nitrogen. O. C. Wilson<sup>1</sup> has used Wright's measurements of the nuclear bands in his discussion of the physical characteristics of the Wolf-Rayet stars. Wright had called attention to the red shift of some of the bands and had attributed it to the encroachment of the absorption components on the violet edges. Wilson has also advanced an alternative explanation based upon the interpretation of the red shift as a gravitational effect, and has stressed the importance of wave-length measurements in this object.

We have recently found<sup>2</sup> a second object, HD 167362, which, like BD +30°3639, shows the rather peculiar association of an exciting Wolf-Rayet nucleus without any trace of nitrogen with a surrounding nebulosity which is very rich in nitrogen. This star has also fairly narrow emission bands. We secured in September, 1939, several slit spectrograms of Campbell's ob-

TABLE 1  
EMISSION LINES DUE TO THE NUCLEUS OF BD 30°3639

λ	INT.	IDENTIFICATION			VIOLET EDGE	RED EDGE	λ WRIGHT	λ PLASKETT	NOTES
		ELEMENT	λ	INT.					
3332.8	2	O III	3333.00	4					
		O III	3330.40	4					
3340.1	3	O III	3340.74	6			3342.		
3347.9	1	O IV	3348.08	2					
		O IV	3349.11	3					
3359.4	1	.....	.....	..					
3375.5	1	O IV	3375.5	2					
3380.2	1	O IV	3381.3	4					
3385.7	1	O IV	3385.6	5					
		O III	3384.95	4					
3391.0	2	O II	3390.25	8					
3396.6	2	O IV	3396.8	2					
3404.1	3	O IV	3403.58	3			3404.		
3412.9	4	O IV	3411.76	4			3414.		
3429.1	2	O III	3428.67	3			3429.		
		O III	3430.60	4					
3445.4	3nn	O III	3440.39	4	3439.6	3452.0	3447.		
		O III	3444.10	5					
		O III	3446.73	2					
		O III	3450.94	4					
3456.	1	O III	3455.12	5					
3470.	1	O II	3470.81	8					
3503.7	2nn	.....	.....	..					
3554.	2	He I	3554.5	1					(1)
3561.	2	O IV	3560.42	1					(1)
		O IV	3563.36	2			3564.		
3567.	2	.....	.....	..					(1)
3586.8	1	He I	3587.4	1					
3609.42	5	C III	3609.61	5	3606.1	3612.2	3611.		
		C III	3608.96	4					
3640.0	2n	O III	3638.70	3			3640.		
3665.6	2	.....	.....	..					
3703.91	3nn	O III	3702.75	5			3705.		(2)
		O III	3703.37	5					
		O III	3704.73	3					
		O III	3707.24	6					
		C III	3703.52	2					
		He I	3705.00	3					
3712.29	2	O III	3712.48	2	3711.5	3716.4			
3714.53	3	O III	3715.08	6					
3760.13	5	O III	3759.87	9	3757.0	3763.5	3760.		
3774.02	2	O III	3774.00	6	3772.5	3778.5			
3790.95	2	O III	3791.26	6	3788.4	3793.7	3794.		
3804.14	2	.....	.....	..					
3813.0	1	He II	3813.53						(3)
		O III	3810.96	2					

TABLE 1 (Continued)

## EMISSION LINES DUE TO THE NUCLEUS OF BD 30°3639

$\lambda$	INT.	IDENTIFICATION			VIOLET EDGE	RED EDGE	$\lambda$ WRIGHT	$\lambda$ PLASKETT	NOTES
		ELEMENT	$\lambda$	INT.					
8817.5	1	O III	3816.75	1					(3)
		He I	3819.61	4					
3888.77	5	He I	3888.65	10	3883.2	3894.3	3884E		(4, 5)
		C III	3889.18	4			3894E		
3916.20	4	.....	.....	..					
3919.81	4	C II	3918.98	6	3913.0	3927.2	3915E	3920.2	
		C II	3920.68	8			3920M		
3924.22	2	Si III	3924.44	4			3925E		
		He II	3923.51						
3934.53	1-2	C IV	3934.1						
3951.39	1	PO III	3951.82	0					
3961.66	2	O III	3961.59	8			3962.		
3974.30	1	O II	3973.27	10					
4026.13	3	He I	4026.19	5	4023.2	4035.6	4027.	4026.	(3, 5)
		He II	4025.64						
4031.61	2	.....	.....	..					
4056.58	3	C III	4056.06	5	4052.3	4060.6	4056.7	4056.5	(5)
4070.59	4n	C III	4067.87	9	4065.0	4075.2	4070.	4068.	(3, 4, 5)
		C III	4068.97	10				4071.	
		C III	4070.30	10					
4088.49	2	Si IV	4088.86	10	4085.2	4093.1	4088.9	4089.	(5)
4115.04	3	Si IV	4116.10	8			4113E		
							4115.1M	4117.	
4120.41	3	He I	4120.81	3	4113.2	4133.6	4121.4M		
		C III	4122.05	3					
4128.41	1-2	Si II	4128.05	8			4129.6M	4127.	
4130.74	2	Si II	4130.88	10			4133. E	4130.	
4142.85	1-2	He I	4143.77	2					
4155.94	4	C III	4156.50	4	4152.1	4158.9	4155.4	4155.	
		C III	4152.43	3					
4162.90	3	C III	4162.80	5	4159.7	4166.1	4163.8	4164.	
4168.56	1	He I	4168.97	1					
4176.99	1	.....	.....	..					
4186.44	5	C III	4187.15	10	4182.3	4191.3	4187.7	4186.	(4)
4200.90	2	He II	4199.87	2			4200.5	4200.2	
4213.	1	Si IV	4212.44	3					(6)
4228.64	2	.....	.....	..			4229.6	4229.	
4237.77	1-2	.....	.....	..			4239.		
4253.08	1	O II	4253.98	8n					(6)
4267.60	5	C II	4267.27	10	4260.2	4273.8	4266.9	4267.2	
		C II	4267.02	8					
4316.16	2	O II	4317.16	8			4316.7	4317.	
		C II	4317.42	4					
4325.23	5	C III	4325.70	8	4321.2	4329.9	4325.8	4325.	(5)
4347.40	2	O II	4345.57	7			4347.	4349.	
		O II	4347.43	5					
		O II	4349.43	8					

TABLE 1 (Continued)  
EMISSION LINES DUE TO THE NUCLEUS OF BD 30°3639

λ	INT.	IDENTIFICATION			VIOLET EDGE	RED EDGE	λ WRIGHT	λ FLASKETT	NOTES
		ELEMENT	λ	INT.					
4367.68	4	C III	4368.14	4d			4368.1	4368.	
4376.16	1	O III	4376.15	1n					
		C II	4374.28	5				4379.	
		C II	4376.78	2d					
4382.05	3	.....	.....	..					(3)
4387.69	3	He I	4387.93	3			4385(nn)	4388.1	(3)
		C III	4388.24	2					
4413.95	3	C II	4411.52	5				4413.9	(3)
		C II	4411.20	5					
		O II	4414.89	10			4416.2		
4419.95	2	.....	.....	..				4417.6	
4441.20	4	C IV	4441.81	0			4441.4	4440.5	(7)
4457.21	1nn	.....	.....	..			4457.	{ 4454. 4459.	(8)
4472.27	3	He I	4471.48	6			4472.4	4471.4	(4, 5)
4516.12	4	C III	4516.93	4			4516.6	4516.4	(4)
		C III	4516.02	3					
4542.03	2-3	He II	4541.63	5			4542.7	4542.2	(5)
4553.0	1	Si III	4552.61	9			4555.0	4554.1	
4568.0	1	Si III	4567.83	7			4569.	4567.8	
4593.06	2n	C III	4593.47	2d			4593.9		
		O II	4590.98	9					
		O II	4596.19	8					
4619.22	2n	C II	4618.85	5d			4619.	4620.	
4631.65	2	Si IV	4631.38	3n			4634.	4633.7	
4650.20	10	C III	4647.40	20	4645.0	4654.1	4651.6	4650.6	(4, 5)
		C III	4650.16	19					
		C III	4651.35	18					
4655.33	4	Si IV	4654.14	4n				4656.4	(3)
		C IV	4658.64	5					
		C IV	4656.5						
4665.74	4	C III	4665.90	6			4666.0	4665.	(4)
		C III	4663.53	4					
		C IV	4664.5						
4686.57	4	He II	4685.81	300			4687.1	4686.3	(4, 5)
4704.	1-2	.....	.....	..			4702.2		(5)
4785.7	1	C IV	4785.6				4786.4	4785.5	
		O IV	4783.4	2					

Notes:  
E = edge; M = maximum.  
(1) The separation of these three lines is difficult.  
(2) Very broad band with complex structure; complicated also by the presence of a line of nebular origin.  
(3) Measurement difficult.

(4) Absorption components measured at: 3882.16 (5); 4063.06 (2); 4180.93 (2); 4465.19 (2); 4510.27 (2); 4641.74 (7, from 4637.82 to 4645.03); 4660.28 (2); 4680.04 (2).

(5) Absorption components measured by Wright at: 3883.0, 4022, 4050.9, 4063.8, 4084.7, 4320.6, 4465.8, 4538, 4641.8, 4681.0, 4695.7.

(6) Line uncertain.

(7) This line is narrower than the C III lines and has a weak violet absorption component.

(8) This very broad line may be double.

ject at the McDonald Observatory. The nuclear lines of BD +30°3639 are relatively narrow and the surrounding nebulosity is not too rich in lines. The star is, therefore, suitable for accurate measurements of wave-lengths. One of the spectrograms was obtained with the 500-mm. camera and the quartz prisms, the dispersion being 40 Å/mm. at  $\lambda$  3933; another quartz spectrogram was taken with a dispersion of 100 Å/mm. at  $\lambda$  3933.

The spectrum has been investigated by Wright,<sup>3</sup> Plaskett,<sup>4</sup> Beals,<sup>5</sup> Edlén<sup>6</sup> and Stoy.<sup>7</sup> Our spectrograms are intended to supplement the earlier material. The sharp lines of nebular origin have been used to correct the radial velocity.<sup>8</sup>

Table 1 contains the results of measurements of the emission lines due to the Wolf-Rayet nucleus. It is limited to the region from  $\lambda$  3330 to  $\lambda$  4786; for the region  $\lambda < 3330$ , we refer to the investigation by Stoy. Above  $\lambda$  4786, our measurements are in good agreement with Wright's table and are, therefore, not reproduced. In the region covered by table 1, Stoy has measured lines at  $\lambda\lambda$  3342, 3358, 3375 and 3385.

The first column of table 1 gives the measured wave-lengths with two decimals when obtained from the spectrogram of higher dispersion; the ultra-violet lines given with one decimal or with no decimal were measured on the spectrogram of low dispersion. Columns 6 and 7 give the edges of some of the lines. Columns 8 and 9 contain the wave-lengths by Wright<sup>3</sup> and by Plaskett.<sup>4</sup> Most of the lines are satisfactorily identified.

Table 1 shows that the nucleus of Campbell's object is a typical carbon star; there is no trace of nitrogen at any stage of ionization.

For the discussion of a possible red shift, the only suitable nuclear features are the strong unblended lines, for which we have good identifications and reliable laboratory wave-lengths and which are not too close to lines of nebular origin. Only the lines measured with the higher dispersion are here considered. About fifteen lines may be used for this purpose. It is obvious that there is no systematic red shift. An appreciable displacement toward the red is observed only in the few cases where the violet absorption component is very strong.

We have also considered our results of measurements of two other planetary nuclei with narrow bands, namely NGC 6543 and HD 167362.



The first Wolf-Rayet nucleus contains N IV and C IV with similar intensities, whereas the second is a pure carbon star. In neither case is there an appreciable systematic red shift.

If we consider the best unblended identifications, we find the following average widths:

TABLE 2		
WIDTHS OF THE BRIGHT LINES OF VARIOUS ELEMENTS		
ELEMENT	IONIZATION POTENTIAL	WIDTH IN KM./SEC.
C <sup>+</sup>	24.26	955
He	24.46	859
Si <sup>+++</sup>	44.95	577
C <sup>++</sup>	47.64	566
O <sup>++</sup>	54.62	474

This table illustrates the well-known decrease in width with increasing ionization potential. The lines do not show an occultation effect, so that the sequence in width corresponds to a sequence in ejection velocity.

A direct determination of the ejection velocity may be made for He I and C III, for which absorption components of the P Cygni type have been observed. The result is: for He I,  $V_{ej.} = 488$  km./sec.; for C III,  $V_{ej.} = 378$  km./sec. This confirms the relation with ionization potential.

\* Campbell's hydrogen envelope star, HD 184738;  $\alpha$  (1900)  $19^h30^m8$ ;  $\delta$  (1900)  $+30^\circ18'$ .

<sup>1</sup> *Ap. Jour.*, **91**, 394 (1940).

<sup>2</sup> *Proc. Nat. Acad. Sci.*, **26**, 454 (1940).

<sup>3</sup> *Pub. Lick Obs.*, **13**, 220 (1918).

<sup>4</sup> *Pub. Dom. Ap. Obs.*, Victoria, **2**, 287 (1924) (especially table 18, p. 348).

<sup>5</sup> *Ibid.*, **4**, 271 (1929).

<sup>6</sup> *Zs. f. Ap.*, **7**, 378 (1933) (see table 9).

<sup>7</sup> *Pub. Astr. Soc. Pac.*, **47**, 162 (1935).

<sup>8</sup> The sharp-line spectrum consists of the usual lines of a low-excitation nebula; the Balmer series is seen to H<sub>22</sub> on our low-dispersion spectrogram.

*GALACTIC AND EXTRAGALACTIC STUDIES, IX. A PHOTOMETRIC SURVEY OF 22,000 GALAXIES IN THE ZONE FROM  $\delta = +41^\circ$  TO  $\delta = +46^\circ$*

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1. *Introduction.*—In an earlier paper a report was made on the space distribution of galaxies in high southern galactic latitude.<sup>1</sup> Radial and transverse density gradients were considered, and an average space-density parameter was derived. The five hundred square degrees covered in that photometric survey are sufficiently near the south galactic pole to leave us essentially free from the difficulties of space absorption.

In the present study we examine the distribution, to photographic magnitude 17.5, and fainter, of the galaxies in a belt which encircles the sky at declination  $+43^\circ.6$ , and is therefore principally in the northern galactic hemisphere. A zone in this particular declination is selected because the plates can be made when the fields are near the Oak Ridge zenith and because the Selected Area sequences,<sup>2</sup> an hour apart in declination  $+45^\circ$ , are available for magnitude work. More than half of the plates contain these standard magnitude sequences, well located for direct use in estimating nebular magnitudes, or for comparison (in galactic latitudes higher than  $20^\circ$ ) with the sequences set up by the star-count method. This direct tie-up all around the zone with a consistent set of standards provides what is perhaps the most homogeneous collection of nebular magnitudes yet obtained.

2. *The Observations.*—The forty-four three-hour photographs used for the survey were made by Mr. Henry A. Sawyer with the 16-inch Metcalf telescope at Oak Ridge during the past five years. The marking of new galaxies and the estimates of photographic magnitudes were nearly all made by Miss Rebecca Jones. The plate material is described in table 1. It will be noted that the belt crosses the galactic plane twice, and that the highest northern galactic latitude attained by the plate centers is  $+74^\circ.0$ , the highest southern,  $-18^\circ.2$ . The material is therefore suitable for examining the galactic latitude effect (space absorption), while still keeping clear of the numerous clusters near the north galactic pole.

In form, table 1 closely follows the first table of Harvard Reprint 194.<sup>1</sup> The coördinates, equatorial and galactic, of the plate centers are for the equinox 1900; the qualities ( $Q$ ) in the sixth column are on the scale of 10 for the highest quality;  $m_s$  and  $m_n$  are, respectively, the plate limit for stars and the limit of nebular completeness, and  $N_{15.5}$  is the total number of objects equal to and brighter than magnitude 15.5.

TABLE 1  
SUMMARY OF PLATES, MAGNITUDE LIMITS AND COUNTS

PLATE	RA (1900)	DEC.	$\lambda$	$\beta$	$Q$	$m_s$	$m_n$	$N_{tot}$	$N_{15.5}$	$N_{15}$
MC 27466	0 <sup>h</sup> 31 <sup>m</sup> 5	+43°56'	88°4	-18°0	6	18.3	17.6	200	5	159
27463	1 01.2	44 04	94.0	-17.8	5	18.2	17.7	301	18	240
29210	1 36.2	43 07	100.7	-17.7	7	18.2	17.5	224	11	163
29232*	2 10.6	43 20	106.9	-15.7	5	18.2	17.5	363	39	275
27881	2 41.1	43 51	111.9	-13.0	7	18.2	17.5	442	30	356
30616*	3 13.0	43 38	117.1	-10.2	7	18.0	17.5	305	59	225
27899†	3 48.1	43 59	122.0	- 6.2	6	17.8		40	1	27
29214	4 15.7	43 13	126.2	- 3.3	6	17.8		25	0	13
29211	4 49.0	43 19	130.1	+ 1.3	5	18.0		12	1	7
27586	5 20.2	43 16	133.5	+ 5.9	4	18.0		16	0	13
27909	5 57.3	44 15	136.1	+12.1	5	18.2		29	2	20
27546	6 30.4	44 01	138.8	+17.4	7	18.6	17.5	185	18	139
28733	7 05.0	44 00	141.1	+23.3	6	18.3	17.7	570	7	468
28753	7 36.8	44 01	142.7	+28.8	6	18.2	17.5	571	10	453
29493	8 15.0	43 39	144.5	+35.5	6	18.0	17.6	711	21	583
29215	8 38.5	44 01	144.4	+39.8	6	18.2	17.7	926	36	716
27687	9 17.9	43 23	145.2	+46.9	8	18.4	17.7	835	35	663
30078	9 47.6	43 47	143.6	+52.2	8	18.5	17.9	1226	41	1028
29265	10 22.1	43 29	141.5	+58.3	6	18.4	17.5	1377	32	1096
29283	10 54.8	43 27	136.8	+63.8	8	18.5	17.5	1016	35	811
28788	11 25.2	43 43	128.7	+68.3	7	18.1	17.5	769	16	594
27639	11 58.1	43 47	115.3	+72.1	9	18.3	17.4	1277	49	974
27704	12 27.6	43 45	98.2	+74.0	5	18.1	17.5	878	43	677
30038	13 04.1	43 42	74.4	+73.6	7	18.0	17.5	1435	31	1172
30129	13 41.8	43 26	55.2	+70.2	6	18.1	17.5	1079	38	890
29962	14 13.4	43 59	46.7	+65.4	6	18.3	17.5	714	19	583
29979	14 42.8	43 51	41.0	+60.8	6	18.1	17.4	639	59	474
28949	15 10.5	43 25	37.2	+56.2	6	18.0	17.3	550	67	415
30079	15 48.9	44 00	36.0	+49.2	7	18.2	17.6	1726	69	1425
30186*	16 25.3	43 34	34.8	+42.7	6	18.0	17.5	1143	50	939
29537	16 54.4	43 48	35.5	+37.5	6	17.9	17.2	500	49	367
30123*	17 26.6	43 29	36.0	+31.6	8	18.3	17.5	808	40	616
30224	17 58.1	43 32	37.5	+26.1	6	17.7	17.2	442	14	373
28950	18 33.9	43 08	39.2	+19.8	6	18.0		222	24	189
30086	19 05.3	43 45	42.1	+14.7	7	17.9		282	23	243
30229	19 38.9	43 27	44.7	+ 9.2	7	17.9		83	10	69
30210	20 10.3	43 29	47.8	+ 4.4	8	17.9		11	1	7
29673	20 46.8	43 22	51.8	- 0.9	5	17.9		8	0	4
29670	21 14.9	43 31	55.4	- 4.4	6	17.9		11	0	9
27412	21 48.4	43 18	59.9	- 8.6	7	18.3		61	5	42
29661	22 19.8	43 02	64.6	-12.0	6	17.9		41	13	30
27880	22 51.3	43 47	70.1	-14.0	6	18.2		59	4	44
29760	23 27.1	43 30	76.3	-16.6	8	18.0	17.4	148	17	108
29768	23 58.0	43 04	81.9	-18.2	6	18.3		51	5	36
					6.4	18.12	17.51	22,311	1047	17,735

\* Contains recognized cluster of galaxies.

† Exposure 155 minutes; all others 180 minutes.

TABLE 2  
SUMMARY OF MAGNITUDES

PLATE	115.5	15.5	15.6	15.7	15.8	15.9	16.0	16.1	16.2	16.3	16.4	16.5
MC 27466	2	3	5	5	10	15	20	25	33	35	36	38
27463	13	14	16	17	22	23	25	27	29	32	36	41
29210	9	10	11	13	15	15	15	16	22	26	30	35
29232	27	31	33	41	46	51	54	59	69	77	85	93
27881	19	23	29	31	37	43	51	59	68	78	85	93
30616	30	38	45	61	67	73	77	79	83	93	102	113
27899	1	1	1	2	6	7	8	10	12	12	13	15
29214	0	0	0	0	0	0	0	0	0	0	0	0
29211	0	1	1	2	2	3	3	4	4	4	4	4
27586	0	0	1	1	2	3	3	4	4	6	6	7
27909	2	2	2	2	2	3	3	4	6	6	9	14
27546	11	13	15	17	21	23	27	31	36	41	44	48
28733	4	4	6	8	11	16	20	24	34	48	59	69
28753	5	6	8	14	21	28	36	46	59	73	87	104
29493	15	16	18	23	32	39	48	56	64	78	89	102
29215	27	33	42	50	55	59	69	80	90	104	116	128
27687	27	29	34	44	56	68	79	90	107	121	134	149
30078	29	32	34	42	55	66	77	87	101	119	136	156
29265	25	27	32	43	59	66	74	82	92	106	121	137
29283	22	29	39	41	49	56	66	77	93	116	137	159
28788	8	12	16	19	26	34	43	52	60	74	86	99
27639	43	49	55	60	70	75	88	99	109	121	140	188
27704	25	30	32	39	45	57	69	81	95	115	129	142
30038	21	25	35	45	55	64	77	93	120	154	177	197
30129	26	28	34	36	44	53	61	69	76	98	113	128
29962	14	16	20	23	29	36	42	49	60	74	89	107
29979	38	42	44	51	56	64	71	77	87	101	111	120
28949	45	51	60	63	68	79	93	107	121	132	148	167
30079	39	48	58	71	86	107	128	146	164	182	200	222
30186	39	46	53	68	91	109	125	141	167	199	225	253
29537	24	31	42	58	71	80	96	112	129	150	164	177
30123	20	25	29	33	45	53	64	76	90	106	123	142
30224	9	13	17	27	40	50	64	80	105	121	138	158
28950	12	18	26	33	38	45	53	62	74	84	94	107
30086	18	21	24	27	32	38	46	56	69	80	94	110
30229	7	9	11	14	17	18	21	26	33	37	42	47
30210	1	1	1	2	2	2	2	3	3	3	3	3
29673	0	0	0	0	0	1	2	3	3	3	3	3
29670	0	0	1	1	1	1	2	3	4	5	6	8
27412	1	1	1	1	4	7	10	13	19	23	25	26
29661	10	11	13	13	14	17	19	21	23	24	24	25
27880	1	2	2	2	2	2	3	5	6	12	15	19
29760	12	14	16	18	22	24	26	29	31	32	34	38
29768	2	3	5	10	12	16	18	18	19	22	24	25
Cum. tot. 44	683	808	967	1171	1438	1689	1978	2281	2673	3127	3536	4016
Log tot. 44	2.83	2.91	2.99	3.07	3.16	3.23	3.30	3.36	3.43	3.50	3.55	3.60
Log tot. 18	2.69	2.76	2.83	2.91	3.00	3.07	3.14	3.21	3.26	3.33	3.39	3.44
Log tot. 9	2.35	2.41	2.49	2.55	2.64	2.70	2.77	2.83	2.90	2.98	3.04	3.11
Non-cum. tot.	683	125	159	204	267	251	289	303	392	454	409	480

16.6	16.7	16.8	16.9	17.0	17.1	17.2	17.3	17.4	17.5	17.6	17.7	17.8	[17.8
43	46	50	60	68	78	84	93	101	117	133	143	152	159
45	49	55	64	71	77	91	105	122	150	171	198	224	240
45	50	55	64	70	76	87	93	102	120	138	149	153	163
104	111	121	130	136	141	156	166	185	213	238	251	266	275
121	135	152	170	189	214	239	253	271	294	315	333	344	356
123	128	135	142	147	153	161	171	185	201	213	219	223	225
16	16	17	20	22	24	27	27	27	27	27	27	27	27
0	0	1	2	3	4	7	9	11	12	13	13	13	13
4	4	4	5	5	6	6	6	6	6	7	7	7	7
8	8	9	9	10	12	12	13	13	13	13	13	13	13
16	17	18	19	19	20	20	20	20	20	20	20	20	20
61	66	72	78	82	88	94	104	110	118	125	130	134	139
82	94	110	128	146	170	203	225	254	291	335	380	426	468
124	140	160	180	196	213	248	272	304	356	391	421	443	453
117	130	146	168	188	213	251	300	352	435	502	561	581	583
141	154	171	191	211	238	284	315	364	431	504	597	664	716
182	205	233	247	263	288	316	341	370	422	472	531	580	663
192	209	228	264	293	325	376	433	486	567	647	732	831	1028
164	177	192	228	268	323	391	470	573	707	808	886	956	1096
185	203	225	264	297	338	403	461	523	607	664	715	758	811
122	140	162	189	214	243	285	332	386	452	508	542	571	594
225	254	291	324	401	467	537	610	701	778	860	919	956	974
176	192	210	233	259	297	336	386	437	495	561	614	653	677
245	272	304	368	426	495	586	679	789	933	1024	1112	1157	1172
158	177	202	236	270	316	388	457	546	637	704	763	825	890
124	145	174	212	239	265	319	360	409	460	499	534	560	583
146	161	179	202	225	254	293	318	354	395	431	457	469	474
186	197	211	233	252	274	312	337	358	387	401	411	414	415
248	269	295	345	392	453	571	685	800	942	1096	1246	1352	1425
294	319	348	391	426	466	532	596	665	774	847	903	933	939
201	213	226	248	266	286	319	338	354	363	365	367	367	367
168	184	204	230	251	274	320	356	402	460	502	554	589	616
183	203	229	259	280	299	331	349	364	371	373	373	373	373
123	134	147	154	162	175	178	183	187	188	188	188	188	189
134	151	172	193	208	223	237	239	243	243	243	243	243	243
53	56	60	63	65	66	69	69	69	69	69	69	69	69
5	5	6	6	6	6	6	7	7	7	7	7	7	7
3	3	3	3	3	3	3	3	4	4	4	4	4	4
8	8	9	9	9	9	9	9	9	9	9	9	9	9
30	32	34	37	39	40	42	42	42	42	42	42	42	42
27	27	28	29	29	30	30	30	30	30	30	30	30	30
22	24	28	32	35	38	39	43	44	44	44	44	44	44
44	48	54	60	65	71	76	86	96	104	106	107	107	108
29	30	32	32	32	32	33	36	36	36	36	36	36	36
4727	5186	5762	6521	7238	8083	9307	10427	11711	13330	14685	15900	16813	17735
3.67	3.71	3.76	3.81	3.86	3.91	3.97	4.02	4.07	4.12	4.17	4.20	4.23	4.25
3.52	3.56	3.60	3.66	3.71	3.76	3.83	3.89	3.95	4.01	4.06	4.09	4.12	4.15
3.19	3.24	3.29	3.35	3.41	3.48	3.55	3.61	3.67	3.74	3.78	3.82	3.84	3.86
711	459	576	759	717	845	1224	1120	1284	1619	1355	1215	913	922

Table 2, in which detailed photometric results are summarized, is analogous to the corresponding tabulation in the earlier paper, except that cumulative totals are given in place of numbers at each tenth of a magnitude. The lines at the bottom of the table give the magnitude frequency (as cumulative totals) directly for all 44 plates, and logarithmically for these 44 as well as for 18 and 9 plates of highest latitude. The last line gives the non-cumulative totals for all the plates.

3. *Discussion.*—An 8 × 10-inch plate made with the Metcalf refractor covers fairly successfully thirty-five square degrees; but, in an analysis of the distribution of galaxies, only the central twenty-five square degrees are commonly used, in order to avoid the uncertain corrections for distance from the plate center that would be necessary if the eastern and western edges of the plate were also included. The loss at these edges can be de-

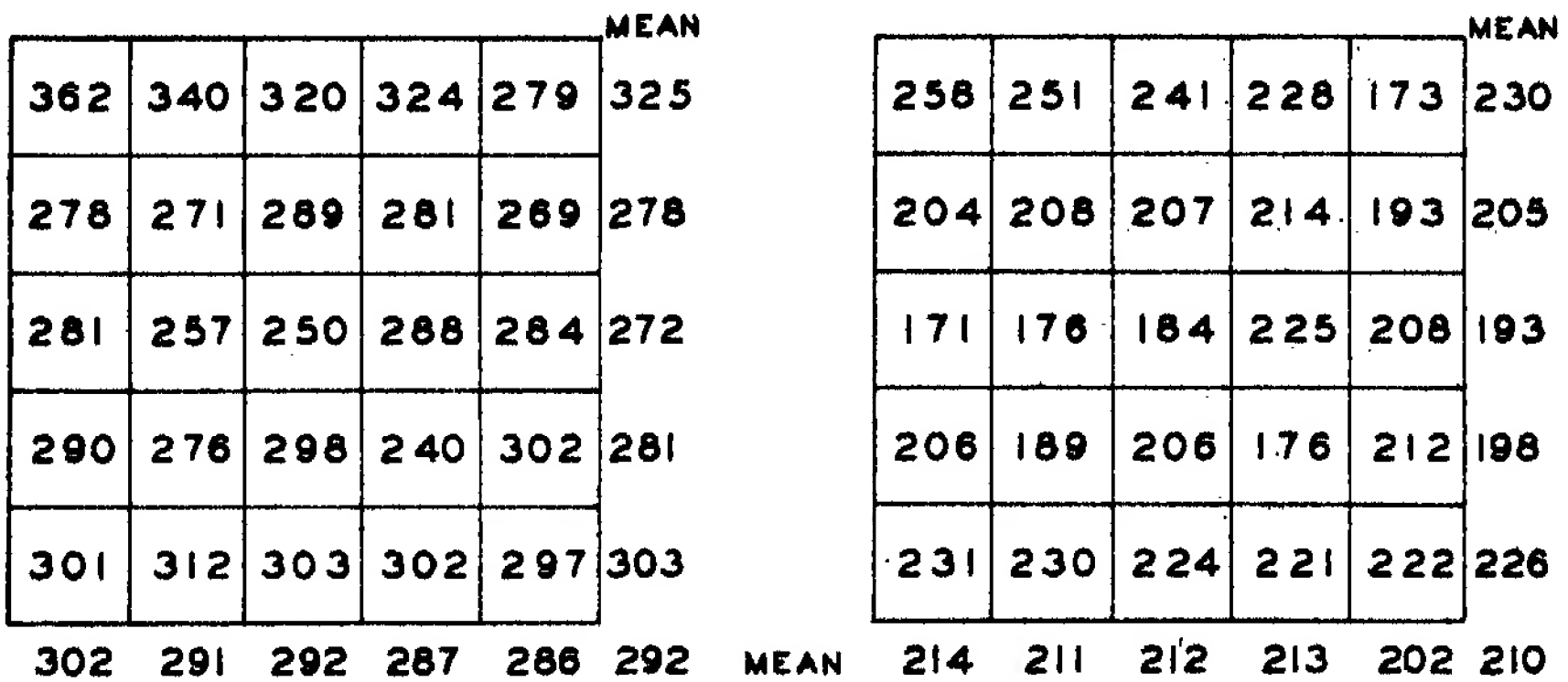


FIGURE 1

Total numbers of galaxies brighter than magnitude 17.1 in each square degree for 44 plates (left), and for 18 plates of highest latitude.

duced from the totals in table 1. Thus for all the plates together there are 16.1 galaxies per square degree for the central twenty-five square degrees, and only 10.4 galaxies per square degree for the strips at the east and west ends of the plate. This difference corresponds, on the average, to an effective loss of nearly one-third of a magnitude; it is partly true magnitude loss, partly failure to discover faint galaxies in an area of slightly distorted images.

To examine possible distance-correction effects within the area of twenty-five square degrees we have constructed figure 1, wherein the results of all 44 plates have been collected in a single checkerboard array, and also separately for the 18 plates with highest galactic latitudes. North is at the top, east on the left. These arrays include, for each of the twenty-five squares, the number of galaxies for which the mean of the twice-estimated magni-



tudes is 17.0 and brighter. Before assembling the arrays, appropriate corrections were made to eliminate from the counts the effect of four nebular clusters (footnote to table 1) that might distort the numbers in a few of the squares. The relatively high uniformity of the plates over an area of twenty-five square degrees results in part from the curving of the plates by air pressure throughout the exposures. Cramer Hi-Speed emulsions, specially sensitized, have been used.

Figure 2 presents a similar pair of checkerboard arrays for objects fainter than magnitude 17.0, again for all forty-four plates as well as separately for those in high latitude. This second pair of arrays reflects as much on the chances of discovery and the completeness of the survey over the plates as on possible distance effects on the magnitude estimates. The deviations from uniformity are of the same order of magnitude as the natural uncertainties. The most conspicuous deviations may be the result of unremoved clusterings; but an examination of the mean values for vertical columns and horizontal lines in the different parts of figures 1 and 2 suggests a slight tilt of the photographic plates, during exposure, about a horizontal axis. There is also an indication in these mean values of a "discovery effect," or a slight systematic magnitude correction, for the corner squares. The difference between corners and central areas is best illustrated by the following tabulation of means, which includes all forty-four plates:

	CENTRAL SQUARE	25 CENTRAL SQUARES	9 CENTRAL SQUARES	4 CORNER SQUARES
To magnitude 17.1	250	292	272	310
Magnitude 17.1 and fainter	402	406	402	374
Total range	652	697	675	684

The result for the eighteen high latitude plates is essentially the same as for the forty-four plates. The difference shown for the total range of magnitudes:

$$\text{Central 9 square degrees} - 4 \text{ corner square degrees} = -9$$

corresponds to an average magnitude gain in the corner square degrees of 0.01, and is therefore negligible. To magnitude 17.1, the difference center *minus* corners of fourteen per cent corresponds to a gain of 0<sup>m</sup>.09, and for the objects fainter than 17<sup>m</sup>.0 there is a loss of 0<sup>m</sup>.04.

Obviously the checkerboard arrays provide a very sensitive means for plate analysis, even when galaxy counts rather than star counts are used. In view of the relative smallness of the derived magnitude deviations, compared with the natural irregularities and the possible uncertainties of the magnitude system, no corrections have been applied to the magnitudes on account of the position of the objects on the plates.

It has seemed advisable, however, to reduce the magnitude estimates by

Miss Jones on the *MC* plates (16-inch Metcalf refractor) to the system of the *A* plates (24-inch Bruce telescope), which cover a much larger part of the sky. The reduction to the Bruce system was carried out before the foregoing examination of the magnitudes was begun; it was simplified by a study of long exposures made by both telescopes on the same rich high latitude fields. Plate *MC* 27639 was measured and the results published by C. K. Seyfert.<sup>3</sup> The magnitudes given by him have been reduced to the Bruce system through the intermediary of measures made by Miss Frances Wright on the same plate.

It will be seen from an inspection of the cumulative totals for each plate, at each tenth of a magnitude from 15.4 to 17.8 (table 2), that the limit of completeness is almost uniformly at 17.5. The limits of the stellar se-

					MEAN						MEAN
378	461	484	489	407	444	323	388	416	430	351	382
364	418	403	432	396	403	322	365	338	382	341	350
337	382	402	394	377	374	275	304	349	346	331	321
415	401	370	440	380	401	340	323	289	377	326	331
374	455	441	421	339	406	306	379	374	374	288	344
374	419	420	435	380	406	313	352	353	382	327	346
					MEAN						

FIGURE 2

Total numbers of galaxies of magnitude 17.1 and fainter in each square degree for 44 plates (left), and for 18 plates of highest latitude.

quences (faintest stars clearly detectable) are much fainter, and in the mean we have, from the data in table 1,

$$m_s - m_n = 0.67 \pm 0.03 \text{ (m. e.)}.$$

For many of the plates in low latitude the population of faint galaxies is too small for dependable estimation of the magnitude to which the survey appears to be complete.

4. *Summary.*—(a) The present paper contains in summarized form information on the apparent photographic magnitudes of some twenty-two thousand external galaxies lying in a declination belt five degrees wide, centered at  $+43^\circ.6$ .

(b) A thousand of the objects are brighter than or equal to magnitude 15.5. The average plate limit for stars is magnitude 18.12; the nebular survey is complete only to magnitude 17.5, but 4400 of the measured galax-

ies within the twenty-five square degrees area are fainter than that magnitude.

(c) The distribution over the plates has been used to examine the nature of the various systematic errors of discovery and measurement. An error arising from plate tilt is intimated; but there is very little suggestion of error from neglected distance correction (figures 1 and 2).

(d) The belt at  $+43^{\circ}.6$  crosses the Milky Way twice, and the results obtained are therefore useful in examining the effect, on the distribution of external galaxies, of interstellar space absorption along the Milky Way. Qualitatively the absorption is clearly shown in the last three columns of table 1. This latitude effect and the magnitude-frequency curves will be more closely studied in a following paper.

<sup>1</sup> These PROCEEDINGS, 26, 166-176 (1940); *Harvard Reprint* 194.

<sup>2</sup> Seares, Kapteyn and van Rhijn, *Mt. Wilson Obs. Papers*, Vol. IV (1930).

<sup>3</sup> *Harv. Ann.*, 105, No. 10, 226 (1937).

## INHIBITION OF CARBOXYLASE BY THIAZOLE PYROPHOSPHATE

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In the course of investigations dealing with vitamin B<sub>1</sub> (thiamin) analogs, and having as their goal the furtherance of our knowledge of the physiological rôle of the vitamin, we have observed that a number of substances having a structure similar to that of cocarboxylase (thiamin pyrophosphate<sup>1</sup>), although not themselves able to replace the latter in the enzymatic decarboxylation of pyruvic acid, are able to inhibit markedly the activity of the carboxylase system. The most active inhibitor studied is the pyrophosphoric acid ester of the thiamin thiazole moiety (the thiazole pyrophosphate portion of the cocarboxylase molecule), which will be referred to as "thiazole pyrophosphate."

The experiments were carried out with the usual Warburg manometric technique. Each vessel contained 0.2 g. dried brewers' yeast which had been washed with alkaline phosphate buffer to remove cocarboxylase. The yeast was suspended in 2.0 ml. 0.2 *M* phosphate (pH 6.2) to which was added 0.5 ml. of 0.1 *M* sodium pyruvate solution (pH 6.2) containing 0.1 mg. Mg<sup>++</sup> and 0.1 mg. Mn<sup>++</sup>. In the side arm of each vessel was placed 1.0 ml. aqueous solution containing 4  $\gamma$  cocarboxylase (synthetic<sup>2</sup>)

and varying amounts of thiazole pyrophosphate; this solution was then tipped into the main part of the vessel after temperature equilibrium had been reached. Measurements were made at 25°C.

The thiazole was pyrophosphorylated by the method<sup>3</sup> which Weijlard and Tauber used in the case of thiamin. The crude material was purified by conversion into the silver salt<sup>4</sup> and from this we obtained a crystalline manganese salt which was used in the experiments tabulated below:

TABLE 1  
INHIBITION OF CARBOXYLASE BY INCREASING AMOUNTS OF THIAZOLE PYROPHOSPHATE  
(MANGANESE SALT)

VESSEL NO.	1	2	3	4	5	6
Thiazole pyrophosphate (Mn salt)	0	4 $\gamma$	8 $\gamma$	16 $\gamma$	32 $\gamma$	80 $\gamma$
Cmm. CO <sub>2</sub> in 30 min.	412	388	385	339	226	41

Free thiazole pyrophosphate, liberated in non-crystalline form from the silver salt, gave results consistent with the above. The inhibition is not due to a change in pH; moreover, pyruvic acid decarboxylation is not affected by free thiazole, thiazole monophosphate or sodium pyrophosphate.

We believe that the observed inhibition phenomenon is to be explained on the basis of a competition between cocarboxylase and thiazole pyrophosphate for the specific carboxylase protein with which the two are similarly able to combine. The introduction, together with cocarboxylase, of a substance also capable of combining with the specific protein but giving an inactive "enzyme analog" results in a lowered rate of pyruvate decarboxylation. This interpretation receives support from experiments in which the thiazole pyrophosphate was not added to the protein simultaneously with the cocarboxylase but at a definite time interval before the addition of the latter. It was found that under these conditions the rate of CO<sub>2</sub> production was initially lower than when coenzyme and inhibitor were added together.

Since it is logical to assume that the protein-inhibitor bond is similar to that between protein and cocarboxylase, this latter must therefore be joined to the protein through the pyrophosphate group,<sup>5</sup> which is common to both it and the inhibitor. Thus we have independent confirmation of the prevailing view<sup>6</sup> regarding the nature of this binding, which view was until now supported almost entirely by analogy with the alloxazine-proteid complex.<sup>7</sup>

We conclude that there has been demonstrated here a not hitherto recognized type of competitive inhibition of enzyme reactions, caused by competition not between substrate and inhibitor but between coenzyme and inhibitor. The importance of this type of inhibition must be emphasized because its study may throw light on structural relationships in protein chemistry and on enzyme reaction kinetics and also because there is the

possibility that such inhibition mechanisms are of significance in the chemistry of the cell.

The authors wish to express their gratitude to the Research Corporation for financial aid and to Dr. R. T. Major of Merck and Co., Inc., for generous gifts of cocarboxylase and other chemicals.

<sup>1</sup> Lohmann, K., and Schuster, P., *Biochem. Z.*, **294**, 188 (1937).

<sup>2</sup> Weijlard, J., and Tauber, H., *Jour. Amer. Chem. Soc.*, **60**, 2263 (1938).

<sup>3</sup> We are indebted to Mr. J. Weijlard (Merck and Co., Inc.) who kindly communicated to us the results of unpublished experiments on the pyrophosphorylation of thiazole.

<sup>4</sup> Lohmann and Schuster (ref. 1, page 196) have obtained the silver salt of thiazole pyrophosphate after cleavage of cocarboxylase by sulfite.

<sup>5</sup> A binding through the agency of some other grouping in the cocarboxylase molecule is also to be expected.

<sup>6</sup> See Stern, K. G., and Melnick, J. L., *Jour. Biol. Chem.*, **131**, 610 (1939); Bersin, T., *Kurzes Lehrbuch der Enzymologie*, page 96, Akademische Verlagsgesellschaft, 1938.

<sup>7</sup> Kuhn, R., and Rudy, H., *Ber. Deut. Chem. Ges.*, **69**, 2563 (1936).

## ON HOMOTOPY GROUPS

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1. We shall denote by  $K$  a finite polyhedron with a fixed cell-decomposition and an orientation attached to each cell.  $K^m$  will stand for the subpolyhedron consisting of all cells of  $K$  of dimension  $\leq m$ . By definition  $K^{-1} = 0$ .

Let  $G$  be an abelian group and  $Y$  a topological space. A triple  $(K, A^n, f)$ , where  $A^n$  is an  $n$ -chain in  $K$  with coefficients in  $G$  and  $f$  is a continuous mapping  $f(K) \subset Y$ , is called a *continuous  $n$ -chain* in  $Y$  with coefficients in  $G$ . Such  $n$ -chains and  $(n+1)$ -chains may serve to define a homology group  $\mathfrak{H}^n(Y, G)$ .<sup>1</sup>

2. Let  $y_0$  be a fixed point of  $Y$ . An  $n$ -chain  $(K, A^n, f)$  will be called an  $(n, m)$ -chain if  $f(K^{m-1}) = y_0$  where  $0 \leq m \leq n$ . Using the  $(n, m)$ -chains and the  $(n+1, m)$ -chains we can define a new homology group  $\mathfrak{H}^{n, m}(Y, G)$ .

In the definition of  $\mathfrak{H}^{n, m}$  it is essential to specify the point  $y_0$ . However, if  $y_1$  is any point which may be joined by an arc to  $y_0$ , then the resulting group is isomorphic with the initial group.

3. Let  $\pi_i(Y)$  be the  $i$ th homotopy group<sup>2</sup> of  $Y$  with the point  $y_0$  as origin ( $i > 0$ ). It is convenient to introduce the notation  $\pi_0(Y) = 0$  to indicate that  $Y$  is arcwise connected. It is well known that if  $\pi_i(Y) = 0$  for  $i < m$



then every mapping  $f(K) \subset Y$  is homotopic to a mapping  $f_1(K) \subset Y$  such that  $f_1(K^{m-1}) = y_0$ . In particular, it follows that every continuous  $r$ -chain in  $Y$  is "homotopic" to an  $(r, m)$ -chain in  $Y$ . Hence

**THEOREM I.** *If  $\pi_i(Y) = 0$  for  $i < m$  then  $\mathfrak{H}^{n, m}(Y, G)$  and  $\mathfrak{H}^n(Y, G)$  are isomorphic.*

As particular cases we obtain:

- (1)  $\mathfrak{H}^{n, 0}(Y, G)$  and  $\mathfrak{H}^n(Y, G)$  are isomorphic.
- (2) If  $Y$  is arcwise connected then  $\mathfrak{H}^{n, 1}(Y, G)$  and  $\mathfrak{H}^n(Y, G)$  are isomorphic.
- (3) If  $\pi_i(Y) = 0$  for  $i < n$  then  $\mathfrak{H}^{n, n}(Y, G)$  and  $\mathfrak{H}^n(Y, G)$  are isomorphic.

4. Take, now, the integers  $T$  as the coefficient group. Let an  $(n, n)$ -chain  $A^n = (K, A^n, f)$  be given where

$$A^n = \sum_i \alpha_i \sigma_i^n \quad \alpha_i \text{ integers.}$$

Since  $f(K^{n-1}) = y_0$  the boundary of the oriented  $n$ -cell  $\sigma_i^n$  is mapped into  $y_0$ , the mapping  $f(\sigma_i^n)$  determining therefore uniquely an element  $d(f, \sigma_i^n)$  of  $\pi_n(Y)$ . Set

$$d(A^n) = \sum_i \alpha_i d(f, \sigma_i^n).$$

For  $n > 1$ ,  $d(A^n)$  is an element of  $\pi_n(Y)$ , for  $n = 1$  the fundamental group  $\pi_1(Y)$  may not be abelian and  $d(A^1)$  is defined only modulo the commutator subgroup of  $\pi_1(Y)$ . If  $A^n$  is the boundary of an  $(n+1)$ -cell of  $K$  then it is clear that  $d(A^n) = 0$ . Hence  $d(A^n) = 0$  if  $A^n$  bounds in  $K$ , and  $d$  is a homomorphic mapping of  $\mathfrak{H}^{n, n}(Y, T)$ .

**THEOREM II.** *For  $n > 1$  the operation  $d$  establishes an isomorphism of  $\mathfrak{H}^{n, n}(Y, T)$  and  $\pi_n(Y)$ .*

*For  $n = 1$  the operation  $d$  establishes an isomorphism of  $\mathfrak{H}^{1, 1}(Y, T)$  and the factor group of  $\pi_1(Y)$  by its commutator subgroup.*

Using (2) and (3) we deduce from Theorem II the following two known results:

(4) *If  $Y$  is arcwise connected then  $\mathfrak{H}^1(Y, T)$  is isomorphic to the factor group of  $\pi_1(Y)$  by its commutator subgroup.*

(5) *If  $\pi_i(Y) = 0$  for  $i < n$  ( $n > 1$ ) then  $\mathfrak{H}^n(Y, T)$  and  $\pi_n(Y)$  are isomorphic.<sup>3</sup>*

5. In view of Theorem II we may denote for  $n > 1$  the group  $\mathfrak{H}^{n, n}(Y, G)$  by  $\pi_n(Y, G)$  and call it the  $n$ th homotopy group of  $Y$  with coefficients from  $G$ . These groups do not furnish any essentially new invariants of  $Y$  as they can be computed using  $\pi_n(Y)$  and  $G$ .<sup>4</sup>

6. The groups  $\mathfrak{H}^{n, m}$  can also be defined relative to a subset  $Y'$  of  $Y$  which contains  $y_0$ .

<sup>3</sup> The mechanism defining this group is quite analogous to the one used by N. E. Steenrod, "Regular Cycles of Compact Metric Spaces," *Ann. Math.*, 41 (1940), in print.

<sup>4</sup> W. Hurewicz, *Proc. Akad. Amsterdam*, 38, 112-119 (1935).



<sup>3</sup> W. Hurewicz, *Ibid.*, **38**, 521-528 (1935).

<sup>4</sup> For other generalizations of the homotopy groups see N. Aronszajn, *C. R. (Paris)*, **202**, 1475-1478 and 1643-1645 (1936).

## ON A GENERAL RULE CONCERNING THE HALF-VALUE PERIODS OF ELECTRON-EMITTING NUCLEI

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In an earlier paper<sup>1</sup> the author has pointed out some periodicities in the properties of stable nuclei. In the following it will be shown that some periodicities also reveal themselves if we consider the half-value periods of unstable electron ( $\beta^-$ )-emitting nuclei.

A table which was recently published by Livingood and Seaborg<sup>2</sup> contains more than 300 unstable nuclei. Among them are 93 which are classified as emitting  $\beta^-$ -particles (without positrons) and as belonging either to class *A*, i.e., isotope and element certain, or at least to class *B*, i.e., element certain, isotope probable. To these 93 nuclei are to be added about seven more, the discovery of which has been published only since January, 1940.<sup>3</sup>

We may now arrange these unstable  $\beta^-$ -emitters in series according to their "isotopic number." By the latter the difference  $N - P$  or, what amounts to the same,  $A - 2Z$  is understood where  $N$  is the number of neutrons,  $P$  of protons,  $A$  the mass number and  $Z$  the charge number. We shall, however, distinguish between such series for which the charge number is even and such for which the charge number is odd. Table 1 contains all sequences of two or more consecutive  $\beta^-$ -emitters of classes *A* and *B*; only those  $\beta^-$ -emitters are omitted for which we know neither a preceding nor a succeeding nucleus ( ${}_{Z-2}X^{A-4}$  or  ${}_{Z+2}X^{A+4}$ ) belonging to class *A* or *B*.

From table 1 we may formulate the general rule that in each sequence the half-value period increases from place to place. Although table 1 contains some 60 nuclei, only four nuclei do not fit into the scheme of this rule, namely  ${}_{11}\text{Na}^{24}$  ( $I = 2$ ),  ${}_{21}\text{Sc}^{48}$  ( $I = 6$ ),  ${}_{28}\text{Ni}^{68}$  ( $I = 7$ ), perhaps  ${}_{49}\text{In}^{112}$  ( $I = 14$ ), and finally in the high sequences  $I = 22$  and 36 the nucleus with longer life precedes that with shorter life, in contradiction to the rule.

We find as another rule that those  $\beta^-$ -emitters for which both indices, i.e., charge and mass numbers, are odd, would be transformed into stable

nuclei by addition of a proton. The only possible exception to this rule is the  $\beta^-$ -emitter  $^{139}_{55}\text{Cs}$  since the barium isotope  $^{140}_{56}\text{Ba}$  is not known, although it might be suspected for other reasons.<sup>4</sup>

TABLE 1  
SEQUENCES OF  $\beta^-$ -EMITTERS WITH THE SAME ISOTOPIC NUMBER

$I = 2$ (odd)	$^5\text{B}^{12}(A)$ 0.022 s	$^7\text{N}^{12}(A)$ 8 s	$^9\text{F}^{20}(A)$ 12 s	$^{11}\text{Na}^{24}(A)$ 14.8 h	$^{13}\text{Al}^{28}(A)$ 2.4 m	$^{15}\text{P}^{32}(A)$ 14.30 d	$(^{17}\text{Cl}^{36}(A))$ >1 y
$I = 2$ (even)	$^3\text{He}^4(A)$ 0.8 s	$^4\text{Be}^{10}(A)$ 380 y	$^6\text{C}^{14}(A)$ very large				
$I = 3$	$^8\text{O}^{19}(A)$ 31 s	$^{10}\text{Ne}^{23}(A)$ 40 s	$^{12}\text{Mg}^{27}(A)$ 10.2 m	$^{14}\text{Si}^{31}(A)$ 170 m	$^{16}\text{S}^{35}(A)$ 88 d		
$I = 4$	$^{17}\text{Cl}^{35}(B)$ 37 m	$^{19}\text{K}^{42}(A)$ 12.4 h	$^{21}\text{Sc}^{46}(A)$ 85 d				
$I = 5$	$^{18}\text{Ar}^{41}(A)$ 110 m	$^{20}\text{Ca}^{46}(A)$ 180 d					
$I = 6$	$^{21}\text{Sc}^{48}(A)$ 44 h	$^{23}\text{V}^{52}(A)$ 3.9 m	$^{25}\text{Mn}^{56}(A)$ 2.59 h	$^{27}\text{Co}^{60}(A)$ 7 y			
$I = 7$	$^{23}\text{Ti}^{51}(A)$ 2.9 m	$^{24}\text{Cr}^{55}(A)$ 1.6 h	$^{26}\text{Fe}^{59}(A)$ 47 d	$^{28}\text{Ni}^{63}(A)$ 2.6 h			
$I = 8$	$^{29}\text{Cu}^{66}(A)$ 5 m	$^{31}\text{Ga}^{70}(A)$ 20 m	$(^{33}\text{As}^{74}(A))$ 17 d				
$I = 12$	$^{33}\text{As}^{78}(A)$ 65 m	$^{35}\text{Br}^{82}(A)$ 34 h	$^{37}\text{Rb}^{86}$ ? ?	$^{39}\text{Y}^{90}(A)$ 60 h			
$I = 13$	$^{35}\text{Br}^{83}(A)$ 65 m	$^{37}\text{Rb}^{87}(A)$ very large					
$I = 14$	$^{45}\text{Rh}^{104}(A)$ 44 s	$^{47}\text{Ag}^{108}(A)$ 2.3 m	$^{49}\text{In}^{112}(B)$ 72 s or 2.7 d				
$I = 15$	$^{45}\text{Ms}^{101}(A)$ 9 m	$^{48}\text{Rh}^{109}(B)$ 46 d					
$I = 16$	$^{47}\text{Ag}^{110}(A)$ 22 s	$^{49}\text{In}^{114}(B)$ 48 d					
$I = 17$	$^{42}\text{Mo}^{101}(B)$ 24 m	$^{44}\text{Ru}^{105}(B)$ 20 h					
$I = 19$	$^{46}\text{Pd}^{111}(A)$ 17 m	$^{48}\text{Cd}^{115}(A)$ 2.5 d					
$I = 20$	$^{51}\text{Sb}^{122}(A)$ 2.8 d	$^{53}\text{I}^{126}(A)$ 13.0 d					
$I = 25$ (even)	$^{50}\text{Sn}^{125}(B)$ 9 m	$^{52}\text{Te}^{129}(A)$ 70 m					
$I = 25$ (odd)	$^{51}\text{Sb}^{127}(A)$ 80 h	$^{53}\text{I}^{131}(A)$ 8.0 d					
$I = 27$	$^{53}\text{Te}^{131}(A)$ 25 m	....	$^{56}\text{Ba}^{135}(A)$ 86 m				
$I = 32$	$^{67}\text{Ho}^{166}(B)$ 85 h	$^{69}\text{Tm}^{170}(A)$ 105 d					

It might be noteworthy that some regularity reveals itself also in the case of the series  $I = \text{minus } 1$ , which consists of positron-emitters. As is seen from table 2, the half-value periods decrease monotonically with the charge number up to Si (Na and Al being missing), irrespective whether the charge number is even or odd.

TABLE 2

THE SERIES I = -1, CONTAINING  $\pi$ -EMITTERS

${}^6\text{C}^{11}(A)$	${}^7\text{N}^{13}(A)$	${}^8\text{O}^{15}(A)$	${}^9\text{F}^{17}(A)$	${}_{10}\text{Ne}^{19}(A)$	${}_{12}\text{Mg}^{23}(A)$	${}_{14}\text{Si}^{27}(A)$
21.0 m	9.93 m	126 s	70 s	20.3 s	11.6 s	3.7 s

<sup>1</sup> Haas, A. E., *Proc. Nat. Acad. Sci.*, **26**, 305 (1940).<sup>2</sup> Livingood, J. J. and Seaborg, G. T., *Rev. Mod. Phys.*, **12**, 30 (1940).<sup>3</sup> Walke, H., *Phys. Rev.*, **57**, 163 (1940); **57**, 177 (1940); Pollard, E., *Ibid.*, **57**, 241 (1940); Seaborg, G. T., Livingood, J. J., and Kennedy, J. W., *Ibid.*, **57**, 363 (1940); Ruben, S. and Kamen, M. D., *Ibid.*, **57**, 549 (1940); Sagane, R., *et al.*, *Ibid.*, **57**, 750 (1940); Amaki, T., *et al.*, *Ibid.*, **57**, 751 (1940); Kennedy, J. W. and Seaborg, G. T., *Ibid.*, **57**, 843 (1940).<sup>4</sup> Haas, A. E., *loc. cit.*

## THE EFFECT OF FAST NEUTRONS ON THE CHROMOSOMES OF TRADESCANTIA

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The development of the cyclotron as a powerful and convenient source of high energy neutrons has made available another type of radiation for use in biological studies. Already several investigations have indicated certain differential effects of neutrons as compared with x-rays and gamma rays on biological materials. In several cases a greater relative effectiveness has been found for a given total amount of ionization produced by the recoil protons resulting from neutron bombardment as compared with an equal amount of ionization produced by other types of radiation, particularly secondary electrons resulting from x-radiation and gamma rays (Lawrence and Lawrence,<sup>1</sup> Zirkle and Aebersold,<sup>2</sup> Zirkle, Aebersold and Dempster,<sup>3</sup> Zirkle and Lampe,<sup>4</sup> Gray and Read,<sup>5</sup> Spear, Gray and Read<sup>6</sup>). In most of these studies rather general physiological responses have been observed. Of particular interest to the cytogeneticist is the effect of radiation on genes and chromosomes. The production of mutations has been demonstrated in *Habrobracon* by Whiting,<sup>7</sup> in *Drosophila* by Nagai and Locher<sup>8</sup> and Timoféeff-Ressovsky and Zimmer,<sup>9</sup> and in mice by Snell.<sup>10</sup> Chromosome aberrations have been produced by Marshak<sup>11,12</sup> in root tips of *Vicia faba*, *Lycopersicon esculentum*, and *Pisum sativum* and by Nishina, Sinotô and Sâtô<sup>13</sup> in root tips of *Vicia faba*. In the present study some preliminary observations of the effect of fast neutrons on the microspore chromosomes of *Tradescantia* are reported.

*Methods.*—The inflorescences from a clone of a diploid *Tradescantia* which has been used in extensive x-ray experiments by Sax<sup>14</sup> were also used in the present investigation. The flowering stalks, kept in cardboard containers, were placed in the beam of neutrons produced by bombarding a beryllium target with 11 Mev deuterons in the Harvard cyclotron. No shielding to exclude radiations other than neutrons was attempted in these preliminary experiments, but certain control experiments which will be discussed later were carried out. The intensity of the radiation was varied by varying the distance of the inflorescences from the target and keeping the time of exposure constant. The ionization produced by the neutrons was measured for each exposure, and readings are given in "n" units, an "n" unit being defined as that amount of ionization produced by neutrons which gives the same reading on a bakelite Victoreen thimble ionization chamber as does one roentgen of x-rays. Following treatment, the flowering stalks were kept in the greenhouse. Observations were made at the first post-meiotic division in the microspore at suitable periods following treatment by the use of aceto-carmin smear preparations.

*Experimental Results.*—The effects of neutrons on chromosomes are qualitatively the same as those produced by x-rays. Observations were made at twenty-four to thirty hours and at five days following neutron treatment. At the earlier stage only chromatid aberrations were observed, and at the later, only chromosome aberrations. The configurations were the same as those resulting from chromosome breakage and fusion of broken ends following x-ray treatment as described and diagrammed by Sax.<sup>14</sup>

Quantitatively, however, the results obtained with neutrons differed in two major respects from those obtained by irradiation of developing *Tradescantia* microspores with x-rays. In the first place, a neutron dose of a given intensity in "n" units was much more effective in producing aberrations than an equal dose of x-rays in "r" units. The data on which the comparisons are based are presented in table 1.

Chromatid dicentrics, resulting from breakage of the two sister chromatids of a single chromosome followed by fusion of broken ends to produce a dicentric and an acentric fragment, were used as these have been found to show an approximately linear proportionality to dosage in x-ray experiments and to be independent of the time factor. In the neutron experiments also the percentage of breaks has been found to be approximately proportional to dosage (Fig. 1). However, for equal doses of "n" and "r" units, neutrons are about 16 or 17 times as effective in producing aberrations as x-rays.

The second difference between the neutron and the x-ray results was in the shape of the dosage curves for exchange break aberrations. In the case of x-rays it has been shown that both chromatid and chromosome

exchanges increase as the square of the dosage. With neutron treatment, however, it seems clear that both types of exchange aberrations are approximately proportional to dosage. The data for chromatid exchange breaks are presented in table 2.

TABLE 1

THE RELATION BETWEEN NEUTRON DOSAGE AND CHROMATID DICENTRICS; TIME CONSTANT. THE RATIO OF EFFECTIVENESS IN THE PRODUCTION OF CHROMATID DICENTRICS OF EQUAL TOTAL IONIZATION BY NEUTRONS AND X-RAYS. EXAMINED AT 30 HOURS

DOSE IN "n" UNITS	TOTAL <sup>1</sup> CHROMOSOMES	CHROMATID DICENTRICS	% B <sup>2</sup>	% B PRODUCED BY EQUAL X-RAY DOSE <sup>3</sup>	RATIO: NEUTRONS X-RAYS
10.1	2232	85	3.8	0.24	15.8
14.6	2391	155	6.3	0.37	17.0
21.0	2954	265	9.1	0.55	16.5

<sup>1</sup> Figures for total chromosomes which are not multiples of six in this and the following tables indicate occasional microspores with seven chromosomes as a result of non-disjunction of meiosis.

<sup>2</sup> The values for % B (= per cent breaks) given in this and the following tables are averages for several slides and consequently cannot be calculated directly from the figures cited in the tables.

<sup>3</sup> Calculated from the equation  $\% B = (D/45)^{1.1}$  (Sax<sup>14</sup>).

The data for chromosome exchange breaks, including both rings and dicentrics, represent the combined results of two separate experiments, both of which gave similar results. The figures are given in table 3 and the relations between percentage of breaks and dosage for the different break types are plotted in figure 1.

TABLE 2

THE RELATION BETWEEN NEUTRON DOSAGE AND CHROMATID EXCHANGE BREAKS; TIME CONSTANT. EXAMINED AT 30 HOURS

DOSE IN "n" UNITS	TOTAL CHROMOSOMES	EXCHANGES	RINGS	TOTAL	% B*
10.1	2232	32	4	36	3.2
14.6	2391	50	8	58	4.9
21.0	2954	98	14	112	8.0

\* Each aberration involves two breaks.

In order to exclude the possibility that slow neutrons were producing the effects observed, a control experiment was carried out. Two similar sets of inflorescences were used, but one was completely surrounded by sheet cadmium 0.106 cm. thick to filter out the slow neutrons. The two sets were placed at equal distances from the target and irradiated at the same time. The results are shown in table 4.

TABLE 3

THE RELATION BETWEEN NEUTRON DOSAGE AND CHROMOSOME ABERRATIONS; TIME CONSTANT. EXAMINED AT 5 DAYS

DOSE IN "n" UNITS	TOTAL CHROMOSOMES	DICENTRICS	RINGS	TOTAL	% B*
10.1	3384	32	12	44	2.6
13.5	3617	62	26	88	4.9
14.6	3497	57	20	77	4.4
21.0	3678	76	22	98	5.3
27.0	3604	86	36	122	6.8
32.3	4783	163	42	205	8.6
51.0	4313	200	66	266	12.5

\* Each aberration involves two breaks.

TABLE 4

TEST OF THE RÔLE OF SLOW NEUTRONS. CADMIUM FILTER USED TO EXCLUDE SLOW NEUTRONS IN B. EXAMINED AT 24 HOURS

SERIES	TOTAL CHROMOSOMES	CHROMATID DICENTRICS	%B	EXCHANGES	%B*
A	3350	147	4.45	78	4.6
B (Cd)	3064	143	4.7	62	3.9

\* Each aberration involves two breaks.

It can be seen that the percentages of aberrations for the two series are essentially the same, indicating that the fast neutrons rather than the slow ones are producing the effects observed, since a Cd filter of the thickness used removes all thermal neutrons (Livingston and Bethe<sup>16</sup>). In other control experiments no detectable effect on the chromosomes or the division spindles was found when flower buds were exposed to the magnetic field and to the background radiation from the cyclotron chamber.

Technical difficulties prevented any accurate determination of the amount of ionization in the beam due to gamma rays. In the case of the beam produced by the cyclotron of the University of Michigan, Zirkle and Lampe<sup>4</sup> have estimated that the total ionization in tissue due to gamma rays does not exceed twenty per cent. However, there is evidence that the effect of gamma rays on chromosomes is essentially the same as that of x-rays (Muller<sup>16</sup>). Consequently, whatever amount of ionization due to gamma rays is present, it would tend to reduce the magnitude of any differential effect due to neutrons, so that the factor of 16 or 17 is actually a minimum value.

*Discussion.*—It seems probable that the greater effectiveness of neutrons as compared with x-rays in producing chromosome aberrations must be due to the vastly different nature of the ionization produced in tissue by these two types of radiation. Neutrons produce ionization in tissues principally as a result of collisions with materials containing hydrogen with the resulting ejection of protons. These recoil protons produce a



very intense ionization along their paths as compared with that produced by the secondary electrons generated in matter by x-rays. On the average, one ion pair is formed every  $10^{-5}$  cm. along a  $\beta$  particle track and every  $10^{-7}$  cm. along a proton track. The difference in ion density is thus about 100 times as great for the protons per unit distance of path as for secondary electrons.

There is considerable evidence in the case of x-rays which indicates that the biological effects produced are proportional to the total amount of ionization quite independently of the wave-length of the rays which influences the distribution of the ionization (Packard,<sup>17</sup> Muller,<sup>18</sup> Timoféeff-Ressovsky<sup>18</sup>). However, since the ionization density over the effective x-ray range varies only by a factor of about 10, it is not surprising that there is a greater effect in the case of protons with their much greater ionization density. Investigations producing evidence for this greater effectiveness of recoil protons from neutron bombardment have been cited. Similar results with  $\alpha$  particle ionization have been obtained by Zirkle<sup>19</sup> who has shown that the biological effect of this type of radiation on fern spores depends not only on the total amount of ionization but also on the distribution or intensity of this ionization within the cell.

In the present study a given neutron dose in "n" units was found to be from 16 to 17 times as effective in producing chromatid dicentrics as is an equal x-ray dose in "r" units. This particular type of aberration has been found in the x-ray experiments to depend upon "one hit," showing a linear relationship with dosage (Sax<sup>14</sup>). Both threads are broken at the same locus with the subsequent fusion of broken ends to give a dicentric chromatid plus an acentric fragment. In seeking an explanation for the greater effectiveness of neutrons in terms of the difference in ionization density, it is well to keep in mind that all of the chromosome aberrations observed in these experiments result from breakage and subsequent fusion of broken ends in such a way as to produce new and recognizably aberrant configurations. There is good evidence, however, that many breaks are produced by irradiation only to refuse in the original position without resulting in visible aberrations. Consequently, we observe only a fraction, and probably a relatively small one, of the chromosome breaks that actually occur. It seems likely that the greater density of ionization in tissues produced by recoil protons causes a greater number of breaks to occur within certain spatial limits—which are known to play an important rôle in the production of aberrations (Sax<sup>14</sup>)—and consequently permits a greater number of aberrant reunions. It seems probable that only occasionally does a single x-ray hit occur in such a way as to break both sister chromatids at prophase; in most cases only one chromatid is broken and such breaks usually refuse. In the case of recoil protons, however, the greater ionization density should result in more cases where a single hit is effective in breaking both

sister chromatids and thus facilitating their subsequent refusion to produce chromatid dicentrics. It is generally assumed in the case of x-radiation that a single ionization is the "hit" which is effective in producing gene mutations and at least some types of chromosome rearrangements. However, the greater effectiveness of proton ionization in producing chromosome aberrations suggests that more than one ionization may be necessary and the differences in the types of curves obtained with neutrons are more easily explained if an electron or proton path is thought of as the effective "hit."

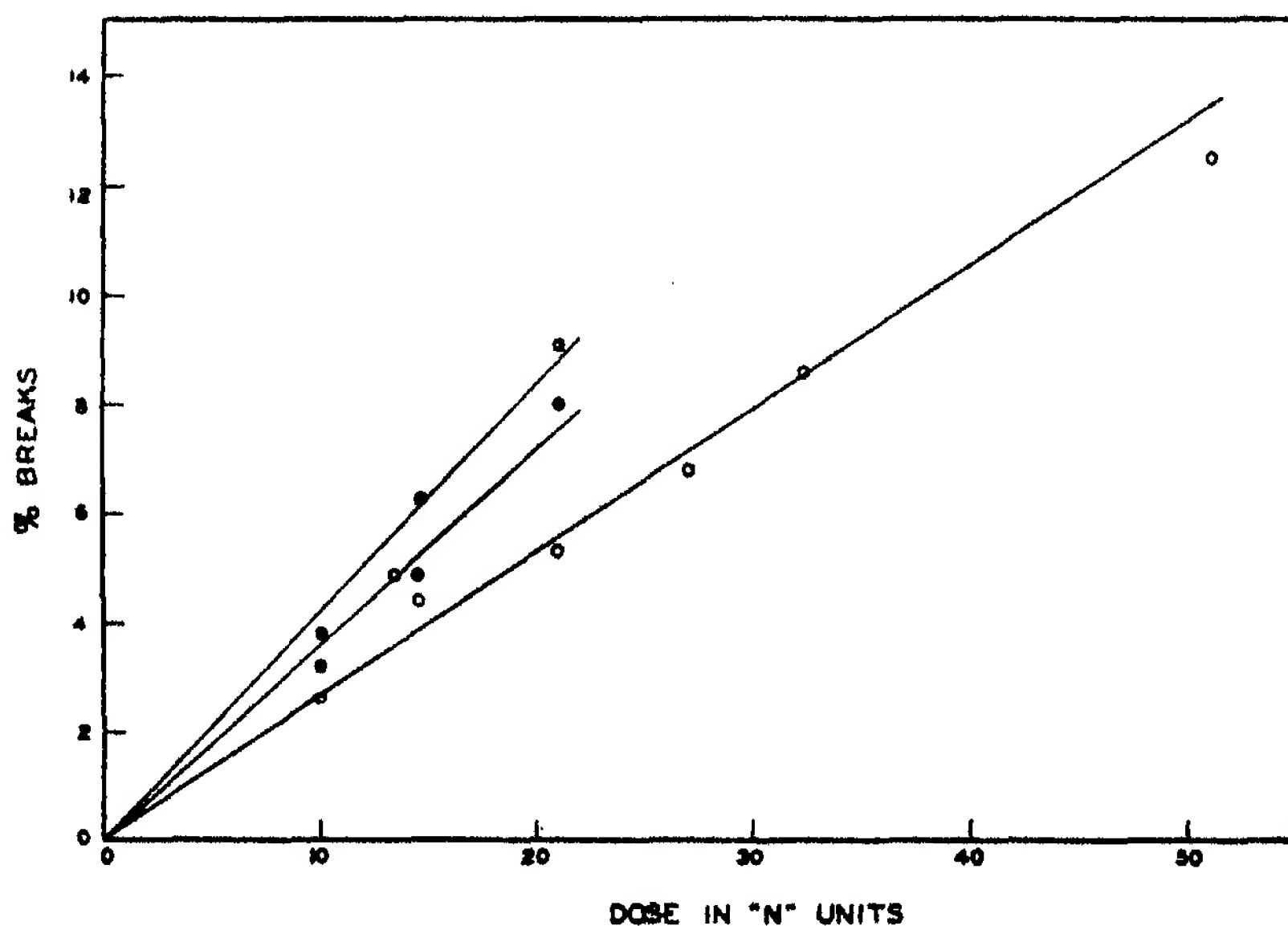


FIGURE 1

Relation between the frequencies of chromatid and chromosome aberrations and neutron dose in "n" units. Time of exposure constant. ● = chromatid dicentrics; ● = chromatid exchanges; ○ = chromosome exchanges.

Presumably an explanation based on differences in density of ionization similar to that given for the dicentric chromatids would also explain the greater number of exchange break aberrations with neutron radiation at the dosages studied. However, it is more difficult to compare exchange breaks as to their frequencies with neutron and x-ray treatment since, as indicated earlier, they appear to show different relationships with increasing dosage—an exponential relation for x-rays and a linear relation for neutrons. At low dosages neutrons are considerably more effective in producing aberrations, but as the dosage increases this greater effectiveness becomes less. Actually there should be some dosage above which

x-rays would be more effective than neutrons. In the present experiments this point is not reached within the limits imposed by the possibility of making a correct analysis of the percentage of aberrations in the treated cells. If the curves are extrapolated to higher dosages, however, this point can be determined. In making the calculations it is assumed, as is pointed out below, that the neutron dosage measurements should be multiplied by a factor of 2.5 to give the actual ionization in tissue as compared with x-ray dosages. When this is done for chromosome exchange breaks it is found that above a dose of approximately 500 "n" units, which is taken as equal to 1250 "r" units, x-rays are more effective than neutrons in terms of equal total amounts of ionization. If this general relation holds for all cells it should mean that above some given dosage, the exact value of which would vary with the kind of cell considered, x-rays would be more effective than neutrons in producing cellular injury as measured by chromosome aberrations. If chromosome aberrations play some part in the regression of tumors following irradiation, these results may mean that above certain dosages neutrons would be less efficient in therapy than x-rays.

It is possible that the apparent greater effectiveness of neutrons over x-rays in producing chromosome aberrations may be due to inaccuracies in the methods of comparing the relative amounts of ionization produced by these two types of radiation in tissue. This particular phase of the problem is being investigated in various laboratories. It is pointed out, however, by Marshak<sup>11</sup> that Aebersold and Anslow have unpublished data which indicate that the "n" unit is not larger than the roentgen by a factor greater than 2.5. If this is the case, ionization from recoil protons is still some 6 to 7 times as effective as ionization produced by x-rays in breaking *Tradescantia* chromosomes.

The experiments of Timoféeff-Ressovsky and Zimmer<sup>9</sup> indicate that neutrons are less effective than x-rays in producing gene mutations in *Drosophila*. However, since all other work so far has indicated a greater biological effectiveness for neutrons, it seems desirable to await confirmation of the mutation results before attempting a comparison of these with the present findings.

The fact that the exchange break aberrations in the case of the x-ray experiments increase as the square of the dosage when the time factor is kept constant has been taken to indicate that these aberrations are dependent upon two independent x-ray hits (Sax<sup>14</sup>). In the present study these aberration types, particularly the chromosome exchanges, show an approximately linear relationship to dosage. This seems to indicate that both the breaks necessary to produce an aberration are the result of a single hit. The difference in the types of ionization paths in tissue of the electrons and protons may explain this difference, a single proton track being capable of producing two breaks. The relation between dosage and

aberrations is not entirely linear for the chromatid exchange breaks, however, and the presence of an exponent greater than one suggests that two independent hits are sometimes involved. More data are necessary to be certain of the relationship. It is also hoped that the time-intensity and intermittent exposure experiments which are being undertaken will provide critical evidence on this point.

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*Summary.*—The effects of fast neutrons on the microspore chromosomes of *Tradescantia* have been investigated and compared with the effect of x-rays. Qualitatively the results are the same as those found after x-ray treatment. Quantitatively, however, neutrons appear to differ considerably from x-rays in their effects on chromosomes. For equal total doses in terms of ionization as measured with a bakelite Victoreen "r"-meter neutrons are from 16 to 17 times as effective as x-rays in producing chromatid dicentric aberrations. Also, exchange break aberrations, producing chromatid and chromosome rings and dicentrics, are found to show an approximately linear relationship to dosage instead of the exponential relation found with x-rays. An attempt is made to explain these differences between neutrons and x-rays as a result of the great difference in the types of ionization paths which these two radiations produce in tissue.

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## CHROMOSOME HOMOLOGIES IN TWO SUB-SPECIES OF *DROSOPHILA VIRILIS*

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It has been shown by Hughes<sup>1</sup> that the two sub-species, *Drosophila virilis virilis* Sturtevant, and *Drosophila virilis americana* Spencer (Spencer<sup>2</sup>), differ, not only in their salivary chromosomes, but also with respect to the metaphase chromosome configuration. The *virilis* metaphase plate shows five pairs of rod-shaped chromosomes and one pair of dots. In *americana*, on the other hand, the female metaphase plate consists of one pair of rods, two pairs of V-shaped chromosomes and a pair of dots, while the male has one pair of rods, one pair of V-shaped chromosomes, a pair of dots, and one V-shaped chromosome typically showing somatic pairing with two rod-shaped chromosomes.

Hughes has concluded that one pair of V-shaped chromosomes in *americana*, the pair found in both sexes, corresponds to two rod-shaped chromosomes in *virilis*; also that the other pair of V-shaped chromosomes found in the *americana* female corresponds to an autosome and the X-chromosome of *virilis*; while the two rod-shaped chromosomes pairing with the V-shaped chromosome in the *americana* male correspond to a *virilis* Y-chromosome and an autosome. (See Fig. 1.)

The following investigation was undertaken to determine genetically the chromosome homologies existing between the two sub-species. More specifically it was desired to secure genetic confirmation of Hughes's cytological findings in regard to the relationships existing between the X, the Y and the autosomes in *americana*.

Reciprocal crosses were made between a stock of *virilis* carrying marker genes on all five major chromosomes, and three strains of *americana*. The three *americana* strains were all collected from points within ten miles of Wooster, Ohio. One of them, the "Smithville" strain, is the one which Hughes used in his cytological investigation.

The *virilis* marker stock carried *y* (yellow) on the X-chromosome, *va* (varnished) on the second chromosome, *tb* (tiny bristle) on the third chromosome, *px*<sup>3</sup> (plexus<sup>3</sup>) on the fourth chromosome and *ru* (ruffled) on



the fifth, the sixth, or dot-shaped chromosome being unmarked. The first series of crosses was between *americana* females and *virilis* males.

$P_1$  *americana* ♀♀ X *y, va, tb, px^2, ru* (*virilis*) ♂♂  
B.C hybrid ♂♂ X *y, va, tb, px^2, ru* (*virilis*) ♀♀

Table 1 gives the types and frequencies of the back-cross offspring.

TABLE 1  
OFFSPRING FROM BACKCROSS OF HYBRID MALE (*americana* ♀♀ X *virilis* ♂♂), X *virilis* ♀♀

PHENOTYPES	SMITHVILLE		OVERTON		PEE WEE-8	
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
<i>y va tb px^2 ru</i>	96		35		73	
wild		135		53		144
<i>y va tb px^2</i>	75		34		88	
<i>ru</i>		108		43		91
<i>va tb ru</i>		121		47		92
<i>y px^2</i>	149		59		164	
<i>y px^2 ru</i>	70		29		42	
<i>va tb</i>		115		50		111

It can be seen that with all three races tested, the back-cross offspring show a complete linkage between *va* and *tb*, thus suggesting that the V-shaped autosomes of *americana* correspond to the second and third chromosomes of *virilis*.

Table 1 shows further that in all cases the females were wild type for

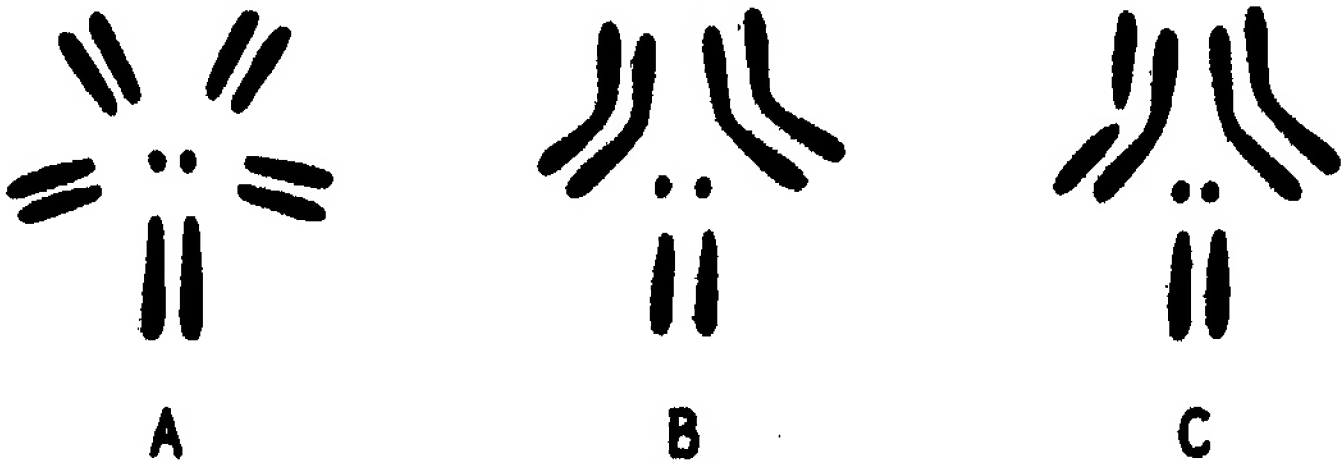


FIGURE 1

A—Metaphase chromosomes of male or female *Drosophila virilis virilis*. B—Metaphase chromosomes of female *Drosophila virilis americana*. C—Metaphase chromosomes of male *Drosophila virilis americana*.

the characters *yellow* and *plexus*<sup>2</sup>, while all the males showed these characters. The sex-linked behavior of *yellow* in the hybrid is in agreement with its location in the X-chromosome of *virilis*. The change from an autosomal inheritance of *plexus*<sup>2</sup> in *virilis* to a sex-linked mode in the hybrid suggests that the *virilis* chromosome carrying *px*<sup>2</sup>, namely the fourth



chromosome, is joined to the *X* to form the V-shaped heterochromosome of *americana*.

A reciprocal cross was made to test genetically Hughes's findings of an *americana* *Y*-chromosome pairing with the *X*, but not, like the *X*-chromosome, attached to an autosome.

*F*<sub>1</sub> *y, va, tb, px<sup>2</sup>, ru (virilis)* ♀♀      *X americana* ♂♂  
*B.C.* hybrid ♂♂      *X y, va, tb, px<sup>2</sup>, ru (virilis)* ♀♀

TABLE 2  
OFFSPRING FROM BACKCROSS OF HYBRID MALE (*virilis* ♀♀ *X americana* ♂♂), *X virilis* ♀♀

PHENOTYPES	SMITHVILLE		OVERTON		PHE WEE-3	
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
<i>y va tb px<sup>2</sup> ru</i>	6	2	23	26	54	62
<i>y</i>	1	4	13	15	103	85
<i>y va tb ru</i>	3	5	16	23	56	52
<i>y px<sup>2</sup></i>	4	5	16	15	105	58
<i>y va tb px<sup>2</sup></i>	2	7	10	23	70	87
<i>y ru</i>	3	5	12	10	13	21
<i>y px<sup>2</sup> ru</i>	3	1	19	20	22	22
<i>y va tb</i>	2	7	26	10	85	89

In this cross the hybrid male received a *Y*-chromosome from the *americana* parent. His offspring did not show sex-linked inheritance of any of the *virilis* autosomal genes. Thus we may conclude that there is no preferential segregation exhibited between the *Y* and any autosome in *americana*, and that the *Y* is not fused to an autosome, thus corroborating genetically Hughes's cytological findings.

In agreement with the data of table 1, the reciprocal crosses reported in table 2 show again for all three races complete linkage between the genes *va* and *tb*, suggesting a fusion of the second and third chromosomes of *virilis* to form the V-shaped autosome of *americana*.

If the chromosomes that are considered to be homologous on the basis of the marker genes used are so with respect to the majority of their genes, the two sub-species might be schematically represented as follows:

*Drosophila virilis virilis*

Female:	(X)	(IV)	(II)	(III)	(V)	(VI)
	(X)	(IV)	(II)	(III)	(V)	(VI)
Male:	(X)	(IV)	(II)	(III)	(V)	(VI)
	(Y)	(IV)	(II)	(III)	(V)	(VI)

*Drosophila virilis americana*

Female:	(X....IV)	(II....III)	(V)	(VI)
	(X....IV)	(II....III)	(V)	(VI)
Male:	(X....IV)	(II....III)	(V)	(VI)
	(Y) (IV)	(II....III)	(V)	(VI)

<sup>1</sup> Hughes, R. D., *Genetics*, 24, 811 (1939).

<sup>2</sup> Spencer, W. P., *Ibid.*, 23, 169 (1938).

### LINKAGE STUDIES OF THE RAT (*RATTUS NORVEGICUS*). III

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A new mutant character of rats, "hereditary acholuric jaundice" was described in 1938 by C. H. Gunn of the University of Toronto. It was shown that the anomaly results from an overproduction of bile pigment in consequence of an excessive fragility of the erythrocytes. The affected individuals are recognizable by their yellow color at or soon after birth. The yellow pigment also enters the developing hair so that white hair of albino and of piebald individuals is distinctly yellowish in color. Growth is usually retarded in affected individuals and in extreme cases "nervous symptoms are developed such as a wobbly gait or partial paralysis, confined chiefly to the hind limbs." Experimental evidence, according to Gunn, indicates that the lag in growth and the nervous symptoms are associated with an inability of jaundiced rats to use carotene as a source of vitamin A, and that they consequently suffer from a prolonged vitamin A deficiency. He shows that the syndrome is inherited as a simple recessive character, which finding is fully conformed by our own observations.

Through the kindness of Professor John W. MacArthur, in whose laboratory the studies of Gunn were made in part, we received in Berkeley a stock of albino jaundiced rats in September, 1938, planning to make a complete study of the linkage relations of the jaundice gene. Dr. MacArthur had already informed us by letter that he had canvassed the question of possible linkage with the commoner mutant rat genes, agouti, albino and hooded, and found no indication of linkage, as recombination occurred freely after crosses involving each of these genes. This finding also we can confirm. The symbol used by MacArthur for jaundiced is *j*, which usage we shall follow.

The mutant genes for which tests have been made by us for linkage with jaundiced are as follows: (1) *A*, agouti; (2) *c*, albino; (3) *Cu*, curly; (4) *Cu*<sub>2</sub>, curly<sub>2</sub>; (5) *d*, dilute; (6), *h*, hooded; (7) *hr*, hairless; (8) *k*, kinky; (9) *wo*, wobbly. Other mutant genes for which no tests have been made because they are known to be linked with one or another of the genes already enumerated are the following: (10) *b*, brown and (11) *an*, anemia,

which are linked with *Cu*; (12) *l*, Grüneberg lethal, (13) *p*, pink-eye, (14) *r*, red-eye and (15) *w*, waltzing, all of which are linked with *c*, albino.

It may be stated at once that all linkage tests made by us have given negative results, so that for the present jaundiced must be regarded as the marker of an independent tenth chromosome.

A summary statement of the results of the linkage tests is contained in tables 1 and 2.

Tests were made by a backcross to the double recessive in the case of agouti, albino, curly, curly<sub>2</sub>, and hooded, the expectation being equality of crossover and non-crossover classes, if no linkage exists. Such equality was found within limits scarcely in any case exceeding the probable error, and so without statistical significance. (See table 1.)

$F_2$  populations (table 2) were considered conclusive evidence of the non-existence of linkage in the cases of dilution, hairless and kinky, in all of which the critical double recessive recombination class, which could arise only from the union of crossover gametes, was found to be close to or in excess of expectation, whereas if linkage existed this class should be below expectation on the basis of free assortment. Also other classes in these  $F_2$  populations were sufficiently close to expectation to negative the idea of linkage.

In the case of wobbly (table 1) an  $F_2$  test was rendered unreliable and a back-cross test between  $F_1$  and the double recessive was rendered impossible by early death of double recessives, none having attained maturity. Accordingly resort was had to the more laborious but more certain method outlined in a previous paper (Castle, 1939). A cross was made between jaundiced and wobbly.  $F_1$  was crossed to animals carrying neither mutant gene. The resulting young were tested individually for presence of one or the other or both of the mutant genes. These tests were made by mating each individual to be tested to an  $F_1$  animal, which from its pedigree would be known to be a carrier of both mutant genes. If there was no linkage between the two mutant genes, those genes would be expected to recombine freely among the gametes produced by an  $F_1$  individual. Such gametes would then be of four sorts equally numerous, *viz.*, (1) *j* only, (2) *wo* only, (3) both *j* and *wo* and (4) neither *j* nor *wo*. No test mating was rated as conclusive unless it resulted in the production of six or more young. Satisfactory tests were made of 143 animals which fall into four classes as follows:

CARRIERS OF <i>j</i> ONLY	CARRIERS OF <i>wo</i> ONLY	CARRIERS OF BOTH <i>j</i> AND <i>wo</i>	CARRIERS OF NEITHER <i>j</i> NOR <i>wo</i>
32	40	23	48

As regards the contribution of the  $F_1$  parent to the animals tested, carriers of *j* only or of *wo* only would arise from repulsion (non-crossover) gametes of the  $F_1$  parent, whereas carriers of *both* or of *neither* would arise from crossover gametes of the  $F_1$  parent. We thus have information as to the nature

of 173  $F_1$  gametes. These total  $32 + 40 = 72$  non-crossover gametes, and  $23 + 48 = 71$  crossover gametes. The two groups are as nearly equal as possible in an odd number of individuals. They furnish a perfect example of free assortment and show conclusively that no linkage exists between  $j$  and  $wo$ .

**Conclusion.**—The recessive mutant gene jaundiced ( $j$ ) shows linkage with no other known rat gene and thus becomes the marker of a tenth independent chromosome pair.

Acknowledgment is made of valuable assistance rendered by WPA laborers in caring for the experimental animals, project No. 65-1-08-91, unit B-7.

TABLE 1  
SUMMARY OF TESTS FOR LINKAGE BETWEEN JAUNDICED ( $j$ ) AND OTHER MUTANT GENES OF THE RAT

BACKCROSSES BETWEEN $F_1$ AND DOUBLE RE- CESSIVES	CROSSOVER GAMETES	NON- CROSSOVER GAMETES	DEVIATION AND P.E.
$j \times$ agouti	87	81	$3 \pm 4.4$
$j \times$ albino	59	59	0
$j \times$ curly	257	267	$5 \pm 7.7$
$j \times$ curly <sub>2</sub>	72	84	$6 \pm 4.2$
$j \times$ hooded	102	113	$5.5 \pm 4.9$
Test by "lethal method," Castle, 1939			
$j \times$ wobbly	71	72	$0.5 \pm 4.0$

TABLE 2 $F_2$ POPULATIONS. EXPECTED 9:3:3:1, IF NO LINKAGE EXISTS				
$j \times$ dilution	$Jd$	$jd$	$Jd$	$jd$
	89	29	22	17
Expected	88.2	29.4	29.4	9.8
$j \times$ hairless	$JHr$	$jHr$	$Jhr$	$jhr$
	95	32	22	9
Expected	89.0	29.7	29.7	9.9
$j \times$ kinky	$JK$	$jK$	$Jk$	$jk$
	352	108	134	51
Expected	362.7	120.9	120.9	40.3

\*This is the third in a series of reports on coöperative investigations of linkage in the rat. In the first two papers all observations reported were made by King at the Wistar Institute. In this paper the observations were made by Castle in Berkeley.

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*SYNTHESIS OF THIAMIN BY EXCISED ROOTS OF MAIZE\**

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Excised roots of many dicotyledonous plants have been cultured indefinitely in a nutrient medium consisting of mineral salts, sugar and one or more accessory growth factors. Some species require the addition of vitamin B<sub>1</sub> (thiamin),<sup>11</sup> others require also vitamin B<sub>6</sub><sup>8</sup> or nicotinic acid or both.<sup>1</sup> According to Robbins<sup>6</sup> the tomato requires only the addition of the thiazole fraction of thiamin but Bonner and Devirian<sup>1</sup> found that thiamin could not be replaced by thiazole for the growth of excised tomato roots.

Similar experiments with monocotyledonous plants have been unsuccessful. Many attempts have been made, particularly with maize (*Zea mays*) and favorable effects have been reported for yeast extract<sup>4, 5</sup> and the extracts of parts of the corn plant or seed,<sup>7</sup> but growth of the tips has not been maintained through more than a few, rarely as many as six, transfers and the rate of growth in the successive transfers has always decreased. Robbins and White<sup>7</sup> and Fiedler<sup>8</sup> found that agar medium was superior to aqueous medium for the growth of excised roots of corn, but a constant growth rate was not maintained through transfers to fresh media and only a few transfers could be made.

Fiedler concluded that it was probably impossible to culture roots of maize indefinitely since it is strictly an annual plant and the growth of the roots is thereby limited. This conclusion is incapable of proof and need not be considered. Robbins and White<sup>7</sup> concluded that organic substances other than glucose as well as minerals not ordinarily supplied were probably essential for the growth of maize roots and the beneficial effect of agar was attributed to impurities that could be assimilated by the roots. Robbins<sup>9</sup> has shown that agar contains traces of growth factors, but the evidence presented herein seems to indicate that the physical properties of the agar solution are, at least in part, responsible for the beneficial effects of the agar.

Evidence is presented in this report that excised roots of maize can be cultured through many transfers without decrease in the growth rate in a solution of mineral salts, glucose and agar, and that under these conditions thiamin is synthesized by the roots.

*Materials and Methods.*—The varieties of maize used in these experiments were inbred lines and single crosses furnished by the Field Crops Department of the University of Missouri. For the most part the single crosses *RYD 940 X L<sub>3</sub>* and *HY X L<sub>3</sub>* were used. The mineral salts used



were Merck's or Baker's c.p. grade, not specially purified. The glucose was cerelose, a pure grade produced by the Corn Products Company. The agar was "Difco, Bacto-agar," granular form. No analysis has been made of the substances and no attempt has been made to purify them. The asparagin was recrystallized four times from hot water. The water was redistilled from Pyrex glass.

The basic mineral solution for the culture of excised root tips was Hoagland's<sup>4</sup> slightly modified, consisting of  $\text{KNO}_3$ —0.25 g.;  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ —0.59 g.;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ —0.246 g.;  $\text{KH}_2\text{PO}_4$ —0.068 g.;  $\text{Fe}_2(\text{SO}_4)_3$ —0.004 g.; water—1 liter. Due to evidence not necessary to this discussion the mineral solution was modified in the later work by the substitution of KCl for  $\text{KNO}_3$  in equivalent parts and the addition of 2 grams per liter of NaCl.

Grains of maize were sterilized in a 0.2% solution of  $\text{HgCl}_2$  in 50% ethyl alcohol for 1 to 2 minutes. They were washed for at least 10 minutes in distilled water in large test tubes and removed to sterile agar plates without nutrients by means of an aluminum spoon. When the roots were from 1 to 4 cm. in length, tips approximately 2 mm. in length were excised and transferred to the nutrient solution.

Unless otherwise mentioned all cultures of both roots and *Phycomyces* were grown in 125-ml. Erlenmeyer flasks of Pyrex glass. The flasks were cleaned with chromic acid cleaning solution, rinsed with tap water, distilled water and finally with redistilled water.

For thiamin analyses the *Phycomyces* method described by Schopfer<sup>10</sup> was used. The medium for the culture of *Phycomyces Blakesleeanus* consisted of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ —0.5 g.;  $\text{KH}_2\text{PO}_4$ —1.5 g.; glucose—100 g.; asparagin—4 g.; water—1 liter. The material to be assayed was ground in a glass mortar and added to 20 ml. of the nutrient medium in 125-ml. flasks. The media were sterilized in an autoclave at 15 lb. pressure for 20 minutes. Each flask was inoculated with 0.2 ml. of a spore suspension made by shaking a tuft of *Phycomyces* in 50 ml. of distilled water. The cultures were allowed to grow for 10 days at a temperature of 20 to 23 degrees in diffuse light. The mycelia were washed with distilled water and dried at 80 degrees.

Estimation of the quantity of thiamin present was made from controls grown with synthetic thiamin ("Betabion, Merck") at the same time and under the same conditions as the assay cultures.

*Experimental Results: Culture of Excised Roots in Aqueous Media.*—All attempts to culture excised corn roots through successive transfers to fresh media have failed. The addition of thiamin, nicotinic acid, amino acids, nucleic acid, ascorbic acid and other growth factors have had little or no effect in any combination tried. Aeration of the medium by bubbling air continuously through the solution was without effect. Although, as is shown later, higher concentration of glucose improves agar media for the



growth of corn roots, 2% glucose was optimum in the aqueous media employed.

*Culture of Roots in Agar Medium with 2 Per Cent Glucose.*—Using Hoagland's solution plus 2% glucose in 1 $\frac{1}{2}$ % agar as the culture medium, the results obtained in this laboratory have been very variable. When the roots were allowed to remain in the original medium for long periods of time, sometimes for 3 or 4 months, they often attained a dry weight of 100 mg. or more, but all attempts to maintain the roots through successive transfers of the tip to fresh media failed. The addition of known growth factors did not improve the solution for the growth of the roots. Extracts of maize seed, leaves or roots of corn, and of yeast were not markedly beneficial even when such extracts were filtered sterile and added to the agar medium at a temperature just high enough to prevent solidification of the agar. It seems probable that the failure of growth under these conditions was not due to the lack of any growth factor that is required in small amounts unless that substance is very unstable or is not absorbed by the roots.

*The Culture of Excised Embryos of Maize.*—It is probable that when an embryo has a large part of the scutellum attached it will not be deficient in thiamin or other required growth factors until it has made considerable growth. Analysis of the seed for thiamin was made for the purpose of determining how much thiamin could be expected to be present under these conditions, and how rapidly the store of thiamin was depleted by the growing plant. Seed of an  $F_1$  hybrid, RYD 940  $\times$   $L_3$ , were soaked for 24 hours at 15 degrees and germinated on agar plates, without nutrients, under sterile conditions, and in the dark at room temperature. Samples were analyzed for thiamin after 0, 5 and 10 days of germination. The results are shown in table 1.

TABLE 1  
ASSAY OF GERMINATING MAIZE SEED FOR THIAMIN

TIME IN DAYS OF GERMINATION	FRACTION OF SEED	THIAMIN, TIMES $10^{-4}$ MOLE
0	Embryo +	
	Scutellum	0.37
	Endosperm	0.02
	Total	0.39
5	Embryo +	
	Scutellum	0.45
	Endosperm	0.02
	Total	0.47
10	Embryo +	
	Scutellum	0.53
	Endosperm	0.02
	Total	0.55

Although the scutellum and embryo constitute less than 12 per cent of the dry weight of the seed they contain about 94 per cent of the thiamin contained in the seed. There was an increase in the total amount of thiamin in the seed; after 5 days the increase was 21.6 per cent and after 10 days 43.2 per cent of the amount originally contained in the seed.

Embryos with approximately  $\frac{1}{4}$  of the scutellum attached were excised from germinating seeds and cultured in the dark in Hoagland's solution plus 0.5, 2 and 5 per cent glucose and  $1\frac{1}{2}$  per cent agar for 21 days. The results are given in table 2.

TABLE 2  
THE GROWTH OF THE ROOTS OF CULTURED EMBRYOS WITH ABOUT  $\frac{1}{4}$  OF THE SCUTELLUM ATTACHED. TIME OF GROWTH WAS 21 DAYS

TISSUE CULTURED	PER CENT OF GLUCOSE	DRY WEIGHT OF ROOTS, MG.
Roots of embryos	0.5	3.0
	2.0	10.8
	5.0	30.6
Excised roots	2.0	9.5

In this experiment glucose was obviously the limiting factor in the lower concentrations. The weight of the roots in the cultures of 5 per cent glucose was more than double the weight of the roots in the lower concentrations. Perhaps more important from the standpoint of this discussion was the fact that in 2 per cent glucose the roots of these plants grew no better than excised roots grown in the same medium. They were similar in appearance, becoming exceedingly thin and stopping growth after about two weeks. In 5 per cent glucose the roots were more nearly normal in appearance and were growing in all cultures at the termination of the experiment after 21 days.

*The Culture of Excised Roots of Maize in Agar Medium Plus 5 Per Cent Glucose.*—On April 15 excised root tips 2 mm. in length were started in the basic mineral solution plus  $1\frac{1}{2}$  per cent agar plus 2, 5,  $7\frac{1}{2}$  and 10 per cent concentrations of glucose. The corn used was a single cross, RYD 940 X L<sub>3</sub>. After 20 days all of the tips in the 2 per cent glucose media were too thin for transferring. Those in  $7\frac{1}{2}$  and 10 per cent glucose were thick but had grown slowly and were discarded. The roots in media containing 5 per cent glucose were normal in appearance and were growing well. These, 8 in number, were sub-cultured by removing 1 cm. of the tip to fresh media of the same constitution. These roots are still growing well at the time of this writing; they are in the eighth sub-culture and have been growing at an almost constant rate of approximately 8 mm. per day for 115 days. Furthermore, each culture, after the tip was removed, developed branch roots that thickened and grew as well as the main root.

In another experiment, that is being carried on at the time of writing, the tips of the roots have been transferred to fresh media at intervals of three days. They have now been transferred 18 times, the rate of increase in length has not varied appreciably and the tips appear to be perfectly normal and as large in diameter as the original tips. During the last 10 of these transfers the tips have been grown in Pyrex petri dishes (150 × 15 mm.) containing 10 ml. of nutrient medium. Many of these roots have at times become abnormal when they penetrated into the agar medium; but growth was resumed at the original rate when the roots were removed from the agar so that only the tip was in contact with the agar or penetration was very shallow.

*Synthesis of Thiamin by Excised Roots of Corn.*—Many assays of excised roots that had grown without transfer in the original medium indicated that in every instance the cultured roots contained more thiamin than was present in the original tip and in the nutrient medium. In each transfer of the experiment described above, started April 15, the old roots were allowed to grow, after the tips were removed, until June 14, when the first four cultures were assayed for thiamin. The results are given in table 3.

TABLE 3  
ASSAY OF EXCISED ROOTS OF MAIZE FOR THIAMIN

TRANSFER	NUMBER OF OF ROOTS	TIME OF GROWTH, DAYS	AV. DRY WEIGHT OF ROOTS, MG.	AV. AMT. OF THIAMIN PER ROOT × 10 <sup>-10</sup> MOLE
1	8	60	62.5	1.81
2	8	30	26.5	0.86
3	7	16	16.3	0.54
4	7	9	7.1	0.33
Total thiamin per root through 4 transfers				3.54
Controls, 1 3-mm. tip excised from a germinating grain of corn, average of 15 cultures				0.15
Culture medium for root culture plus asparagin, average per flask				0.02

A single root growing through 4 transfers was found to contain  $3.54 \times 10^{-10}$  moles of thiamin compared with  $0.15 \times 10^{-10}$  in an original root tip and  $0.02 \times 10^{-10}$  moles in the agar medium. The growth of *Phycomyces* was little better in agar without thiamin than in water without thiamin and evidently only a trace of this substance is to be found in agar. A cultured root contained about 24 times as much thiamin as the original tip and there seems to be no way to account for this increase other than synthesis by the roots from glucose, mineral salts and whatever impurities may be present and available in the medium.

*Discussion.*—No improvement over results already reported has been made in the culture of excised corn roots in aqueous media. The addition of numerous growth factors and extracts prepared in various ways has failed to improve, materially, the mineral salt plus glucose solution and the growth of the excised roots has consistently been unsatisfactory. The results are similar regardless of the organic substances added to the solution and in view of the results reported with agar cultures it seems doubtful that this unsatisfactory growth is the result of a deficiency of any growth factor. It seems more probable that the physical conditions of the aqueous medium are unsatisfactory for the continued growth of excised maize roots.

The conditions under which sustained growth of excised maize roots can be maintained are yet imperfectly delineated. Growth has been maintained through 18 transfers without appreciable decrease in the rate of elongation of the roots and branch roots have been removed from the latest transfers and in turn maintained in culture. It seems doubtful that any substance in the original tip could still be present in sufficient concentration in the later cultures to control the growth of the roots. However, the fact that root tips often become abnormal and cease to grow, even under the most favorable conditions, if they are allowed to penetrate the agar, and that the growth of such roots will often be resumed if they are placed with only the tip in contact with the agar, indicates that conditions other than nutrient deficiency are responsible for the development of the abnormal condition. The fact that maize plants will grow with their roots in water solution does not necessarily invalidate this argument. When the roots are attached to the aerial part of the plant the vascular system is uninterrupted, nutrients are furnished from the aerial parts and there is not usually glucose in the medium surrounding the roots. This is obviously a system quite different from an excised root growing entirely surrounded by the medium. The most plausible explanation of the effect of removing a root from the agar is that aeration is better; and yet it is difficult to understand how a system could be deficient in air when air bubbles are continuously passing through the system, or how a layer of medium only 2 mm. in thickness could be deficient in air. Although it seems likely that physical conditions of the medium are important, the nature of the factors involved are unknown.

The evidence for the synthesis of thiamin by excised roots of maize seems to be conclusive. Whether glucose and mineral salts alone are sufficient for the synthesis of thiamin and for continued growth of the roots is still open to some doubt. It is possible that traces of impurities in the agar and glucose can be utilized. Whatever impurities are present are not effective in improving materially the growth of excised roots in aqueous medium and it is doubtful if their presence is of great importance in the agar

media. It is clear that excised roots of corn can utilize materials that are not effective in replacing thiamin for the growth of *Phycomyces* and convert them into thiamin or its equivalent as measured by the growth of *Phycomyces*.

*Summary.*—1. Efforts to improve an aqueous solution of mineral salts and glucose for the growth of excised maize roots by the addition of known growth factors or of various extracts were not successful. Aeration did not improve the solution for the growth of the roots.

2. Excised maize roots were maintained in a medium consisting of mineral salts, 5% glucose and 1½ per cent agar for 115 days through 18 transfers without appreciable decrease in the rate of elongation of the roots.

3. Thiamin was synthesized in significant quantities by excised roots of maize growing in the mineral salt, glucose and agar solution.

4. Evidence is presented that the physical conditions of the media were probably limiting factors for the growth of maize roots in an aqueous medium and under certain conditions in agar medium.

\* A portion of a dissertation to be presented to the Graduate Faculty of the University of Missouri in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

<sup>1</sup> Bonner, James, and Devirian, Philip S., "Growth Factor Requirements of Four Species of Isolated Roots," *Am. Jour. Bot.*, **26**, 661–665 (1939).

<sup>2</sup> Bonner, James, and Erickson, J., "The *Phycomyces* Assay for Thiamin (Vitamin B<sub>1</sub>): the Method and Its Chemical Specificity," *Ibid.*, **25**, 685–692 (1938).

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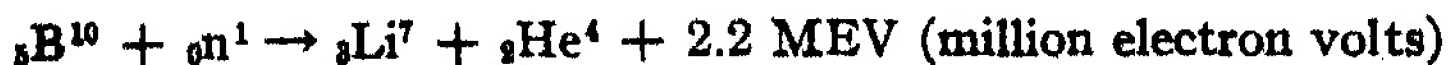
## *SOME IN VIVO EFFECTS OF LOCALIZED NUCLEAR DISINTEGRATION PRODUCTS ON A TRANSPLANTABLE MOUSE SARCOMA*

BY PAUL A. ZAHL,<sup>1</sup> FRANKLIN S. COOPER<sup>2</sup> AND JOHN R. DUNNING<sup>3</sup>

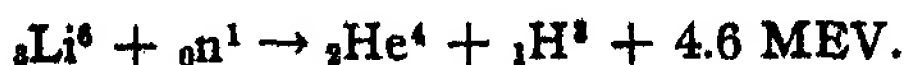
Communicated June 26, 1940

*Introduction.*—The destruction of living tissues by x-ray and fast neutron radiation is well known. The effects are directly traceable to the action of energetic electrons resulting from the absorption of the x-rays in the one case, and in the other, from recoil nuclei, such as those of hydrogen, carbon and oxygen which have been projected by neutral impact. However, in both cases the destructive action occurs throughout the irradiated tissue, and no satisfactory method has been found for localizing the damage, in the case of cancer therapy, to the tumor zone. Thus skin damage usually sets an upper limit to the dose which can be delivered through the skin to underlying tissue.

Since the passage of slow neutrons through body tissues is not accompanied by the production of energetic recoil protons, there should be little or no resulting damage to the tissue. However, if these slow neutrons be introduced into a zone which has been perfused with certain chemical elements such as boron or lithium, or their compounds, nuclear capture reactions will occur which release very energetic particles, and result in the local destruction of tissue. That is, for boron:



or for lithium:



The energies released by these reactions are approximately 2.2 MEV and 4.6 MEV, respectively. The cross-section<sup>4</sup> for the boron process is  $57.5 \times 10^{-24} \text{ cm.}^2$ , as compared with  $60 \times 10^{-24} \text{ cm.}^2$  for the lithium process. Thus while the energy release for lithium is larger, approximately five times the atomic concentration of lithium is necessary to obtain the same total energy release as with boron. Both reactions are competitive with capture

of the slow neutrons by hydrogen, with emission of high energy gamma rays,  ${}_1^1\text{H} + {}_0^1\text{n} \rightarrow {}_1^2\text{H} + \gamma$  (2.2 MEV), but the capture probability for this process is only  $0.3 \times 10^{-24}$  cm.<sup>2</sup>, so only very small atomic concentrations of boron or lithium are required to capture an appreciable fraction of the neutrons.

The foregoing considerations suggest an investigation of the applicability of neutron-boron or neutron-lithium techniques to the localized treatment of tumors. The physical principles have been discussed at length by Kruger (1) in connection with his experimental findings that mammary carcinoma, lymphoma and an undifferentiated sarcoma showed decreased "takes" subsequent to immersion in boric acid solution and *in vitro* irradiation by slow neutrons.

In an effort to test the effectiveness of the neutron-boron process *in vivo*, experiments were designed to attempt to localize boron or lithium atoms within or around the tumor mass of Mouse Sarcoma 180 during the period of irradiation with slow neutrons. This tumor type was considered suitable for such work because of its known radio-sensitivity and its standardizable characteristics.

*Experiments.*---The implantation technique together with the growth characteristics of Mouse Sarcoma 180 have been fully described by Sugiyura (2), *et al.* Suffice it to say that when inoculated subcutaneously in the axillary region with a 2-mm. cube of freshly excised tumor tissue, male mice of 20–22 grams will ordinarily die during the third or fourth week following implantation. The implanted fragment undergoes rapid growth; metastases are not formed. Death presumably is due to impoverishment of the animal by the growing tissue mass, together with a toxemia syndrome resulting from the by-products of necrosis.

The aim was to inject the tumor with various boron or lithium preparations, followed by neutron irradiation of the whole animal with a dose somewhat below that which would ordinarily kill the animal due to general irradiation effects. It was hoped that by taking advantage of the neutron-boron reaction at the site of the tumor, one could develop a local ionization sufficiently intense to destroy the malignant tissue.

The mouse to be irradiated was placed in a small, perforated aluminum shell. Each shell was placed in a paraffin well capped with a paraffin block. For dimensions of these structures see figure 4. The blocks, piled in stacks of three, were placed within the radiation zone of the cyclotron target, in most experiments about forty centimeters from the target.

The neutrons were produced in the cyclotron at Columbia University by bombarding a beryllium target with protons of approximately 7 MEV energy. The neutrons emitted by this reaction have a spectrum rich in neutrons of 0.5 to 2 MEV and comparatively few have energies above 3 MEV.

Many of the neutrons from the cyclotron were slowed down by impacts with the hydrogen nuclei in the paraffin blocks. The thickness of the paraffin walls was a compromise between that necessary to obtain the maximum slow neutron radiation density in the region of the organism to be irradiated and the minimum ionization due to fast neutrons, taking into account the low intensity of the primary neutron beam. One inch of brass (the chamber wall) reduced the gamma radiation from the target, so that the background ionization due to gamma rays in the region of the tumor was less than 20% of the total ionization under these conditions. The increased ionization in a region containing boron is illustrated in figures 1 and 2. Sufficient space was not available for additional lead, but under more favorable conditions, its proper use could reduce the gamma ionization to a still lower value.

Dosages were adjusted from empirical biological observations, aided by existing information on the reaction of mice to known dosages of x-rays. This was necessary, first, because no accurate measure of the energy release of the slow neutron-boron reaction could be made at the tumor site; second, because the biological effectiveness of this energy is not quantitatively understood.

Before undertaking the boron experiments *per se* it was necessary to determine the lethal limit of irradiation in terms of time for the whole animal. Data given by Lawrence, Aebersold and Lawrence (3) indicate that whole-animal x-ray irradiation of 500 roentgens reduces the average life of mice following irradiation to 17 days; 600 r to 12 days; 700 r to 10.5 days; 800 r to 7 days; 1000 r to 5 days. Because of the scatter around each of these mean periods it was arbitrarily assumed that any of our irradiated animals surviving a period of 35 days had escaped the lethal effects of irradiation, and had not been subjected to a total ionization of more than the biological *equivalent* for the mouse of 400 r of x-rays. The validity of this assumption is borne out by Sugiura's (2) findings that 81% of mice receiving 400 r survive indefinitely.

Experiment 1 of table 1 indicates that 18 hours of irradiation in the paraffin wells was 100% lethal. In experiment 2 the irradiation period was reduced to ten hours, and it was found that 66.6% of the animals survived, indicating an approach downward toward the non-killing threshold. From a study of the Lawrence, Aebersold and Lawrence data and that of Sugiura, it was assumed that during somewhat under a ten hour period our animals were being subjected to an effective biological ionization equivalent of between 300 r and 500 r of x-radiation, which according to Sugiura's data is on the borderline between lethal and sub-lethal. The neutron dosage (without the paraffin filter) as read on a standard Victoreen r-meter was approximately 30 "n" per hour.

TABLE 1

TABLE PRESENTING DATA ON MICE SUBJECTED TO VARIOUS TYPES OF TUMOR INJECTIONS TOGETHER WITH SLOW-NEUTRON IRRADIATION									
EXPER. NO.	MATERIAL INJECTED	QUANTITY	RADIATION PERIOD	NO. OF ANIMALS	NO. OF ANIMALS ALIVE AT END OF 35 DAYS	% OF TOTAL LIVING AND SHOWING TUMOR CURE OR REGRESSION	% OF TOTAL DYING FROM TUMOR, GENERAL REGRESSION RADIATION EF- FECTS OR NATURAL CAUSES	% CURE OR REGRESSION DUE TO BORON- NEUTRON PROCESS	
1	No tumor	...	6 hrs. on each of 3 successive days. Total—18 hrs.	Irradiated	18	none*	100.0	..	..
				Non-irradiated	24	21	12.5	..	..
2	No tumor	...	5 hrs. on each of 2 successive days. Total—10 hrs.	Irradiated	18	12	33.3	..	..
				Non-irradiated	24	22	8.4	..	..
	Particulate boron		5 and 6 hrs. on each of two successive days. Total—11 hrs.	Irradiated	12	2	83.4	8.3	..
3	in sesame oil	0.1 cc.		Non-irradiated	12	1	91.7	..	..
3a	None	...	Same as in No. 3	Irradiated	12	1	91.7	..	..
				Non-irradiated	12	2	83.4	..	..
4	Lithium meta- borate in ses- ame oil	0.1 cc. 50 mg. 6 hrs.		Irradiated	24	13	45.9	16.0	..
				Non-irradiated	21	8	61.9	..	..
5	Same as in No. 4	0.05 cc. 0.05 cc. Tot. 50 mg.	3 hrs. on each of two successive days. Total—6 hrs.	Irradiated	18	12	33.4	22.2	..
				Non-irradiated	18	8	55.6	..	..
6	Boric acid in ses- ame oil	0.05 cc. 0.05 cc. Tot. 50 mg.	4.5 and 3.6 hrs. on two successive days. Tot. 8.2 hrs.	Irradiated	24	12	50.0	45.0	..
				Non-irradiated	20	1	95.0	..	..
7	Same as in No. 6	0.1 cc. 7 hrs.		Irradiated	18	10	44.5	15.5	..
				Non-irradiated	20	8	60.0	..	..

\* All animals in this experiment were dead within the first 25 days, presumably from general irradiation effects.

Since the appropriate time-dosage period was ascertained grossly to be between six and ten hours, it was necessary to develop a technique for retaining a high concentration of injected material at the tumor site for long periods. Preliminary experiments designed to test the permeability and diffusion characteristics of various forms of boron and lithium indicated: (1) that a saturated aqueous solution of boric acid injected in or around the tumor would not sustain itself in sufficiently high concentration for more than ten or fifteen minutes, as ascertained by making qualitative analyses for boron content of the tissue by the use of the quinalizarine tests of Feigl (4); (2) that finely pulverized metallic boron particles (in the order of 0.5–2.0  $\mu$  in dimension) when injected in oil suspension would localize largely at the oil-tissue interface and would not diffuse through the cell membranes or far into the intercellular spaces of the compact tumor tissue. Since the range of the disintegration products of the neutron capture processes was limited to less than fifty microns, the ionizing effect during radiation would be too local for a general tumor-killing effect. (3) That when powdered boric acid suspended in oil in liquid-paste form (one gram doubly pulverized boric acid suspended in two cubic centimeters of sesame oil) and injected into or around the tumor, a large excess of the boric acid could be localized and remain harmless to the tissue, slowly being taken into aqueous solution by the body fluids bathing the tumor and its environs. Thus a relatively high concentration of aqueous boric acid could be sustained at the tumor site for as long as several hours, draining out of the oil suspension into the soluble water phase. (4) That when lithium meta-borate (which is much less water-soluble than boric acid, and which in aqueous solution hydrolyzes into lithium hydroxide and boric acid) is likewise suspended in oil and injected, it goes into body solution even more slowly than the boric acid oil suspension, and therefore was considered suitable for injection preceding very long radiation periods. The toxicity of these materials will be discussed subsequently.

The exact method of injection is a point which warrants description. The needle was inserted under the skin about an inch posterior to the tumor, then passed subcutaneously into the tumor site and through the center of the tumor mass, and extended into the connective tissue anterior to the tumor. The needle was then slowly withdrawn as the plunger was applied so as to deposit material anterior to, within and posterior to the tumor. Before withdrawing the needle from the skin more material was deposited on either side of the tumor. The purpose, of course, was to bring the actively proliferating tumor tissue into close contact with the boron or lithium-bearing oil.

Having found the time-dosage threshold, it was necessary to establish the independent effects on the tumor of each of several variables. The first was radiation itself without injection. Twelve animals with growing, week-old



tumors were irradiated for eleven hours. Only 8.3% of these survived the 35-day period, the others dying presumably because of the sarcoma growth. In the non-irradiated control to this experiment 16.6% of the animals survived. Since the difference between 8.3 and 16.6, in view of the size of the sample, is hardly significant, it can be assumed that the whole body irradiation did not affect a significant regression of the tumors. This is rather what would be expected in view of the fact that from Sugiura's data it requires an equivalent of 1000 r to effect a 50% *in vivo* regression in shielded animals.

The second variable was the toxicity of the injected materials on both the whole animal and the tissue at the site of the injection. Preliminary experiments indicated that amounts of both boric acid and lithium metaborate could be injected subcutaneously into healthy mice in considerable excess to the 25–50 mg. doses injected for the experiments, without any observable ill effects. However, in experiments 4, 5, 6 and 7 it will be seen that the non-irradiated tumor-bearing mice receiving control injections equal to those in the irradiated specimens showed tumor regression or ab-

#### DESCRIPTION OF PLATE

Figures 1 and 2. Increased ionization produced in regions containing boron when bombarded with neutrons. Fig. 1: Without boron. Eastman Alpha Particle Spectroscopic plate, emulsion No. 129,975. Film exposed to cyclotron irradiation within aluminum shells and paraffin-well in position identical to that occupied by mice in the experiments described in the text. Three tracks due to protons projected by neutrons are clearly visible in the field, together with general photographic grain reduction due to protons projected at various angles to the plane of the film, together with projected nuclei such as carbon, and to gamma-ray background. This photo illustrates essentially the amount of ionizing energy released in an equivalent volume of hydrogen-rich tissue during an equivalent period under conditions described in the text when no boron is present. Magnification:  $\times 1000$ . Fig. 2: With boron. Same type of film and same exposure time as in figure 1, except emulsion before exposure was dipped into a 2% aqueous solution of boric acid and allowed to dry. Shows numerous alpha particle tracks resulting from slow neutron-boron capture. Many tracks do not lie in plane of the photograph. Illustrates essentially the amount of ionizing energy released in an equivalent volume of tissue in the area of the tumor in which an equivalent concentration of boron following injection with boron salts was maintained. Cf. figure 1. It is the ionization differential illustrated in these two photographs which presents the basic rationale of the experiments described in the text.

Figure 3. Cyclotron with paraffin bricks *in situ*. In each brick are two wells of dimensions indicated in figure 4. Loosely placed in each well is a perforated aluminum shell for housing the living mouse during the radiation. The bricks, in stacks of three, were placed in an arc around the beryllium target in a position designed, so far as possible, to equalize the dosage for each animal. Thus twenty-four mice could be irradiated at one time.

Figure 4. Close view of paraffin brick and wells used in all the experiments. A mouse enclosed in the aluminum shell could be retained in relatively comfortable confinement for more than six hours. The wells were closed from above with paraffin slabs loosely fitted for ventilation.



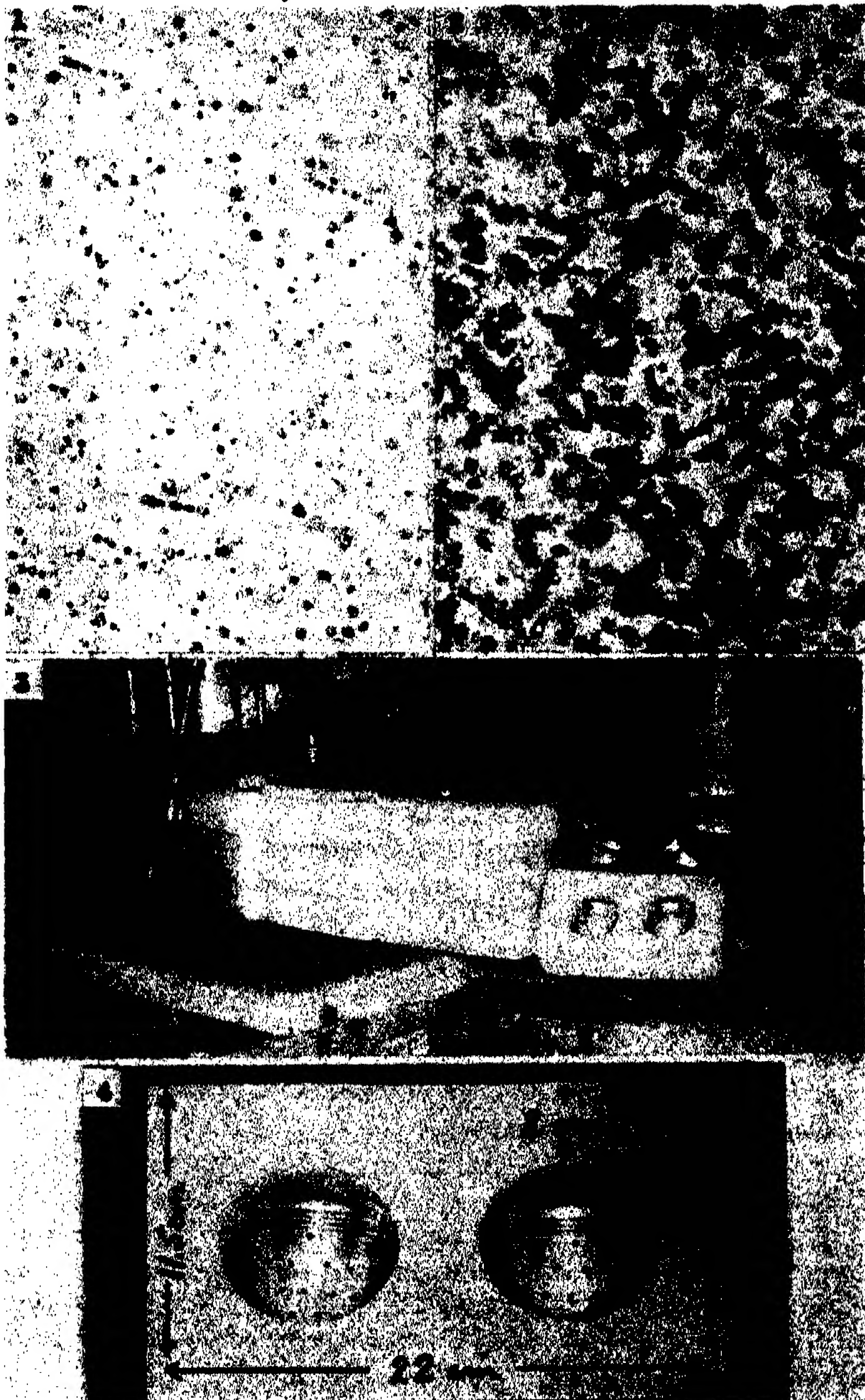


PLATE 1

See opposite page for description of plate.

sorption considerably higher than is normally found in non-injected and non-irradiated tumor-bearing mice. In the four experiments cited the percentages of regressions following injection alone were respectively 38.1, 44.4, 5.0 and 40.0, as compared with the 16% regression in the untreated mice.

This is taken to mean that one or both of the following factors are operative in causing this regression: (1) mechanical injury of the inserted needle to the growing tumor tissue, to the blood channels feeding it or to the connective ground tissue; (2) toxic or destructive effects of the injected materials on the growing tumor tissue, on the blood channels feeding it or on the connective ground tissue. One is inclined to believe that a combination of these two factors is responsible for the increased regression incidence following mere injection of the materials under question.

It is obvious that only percentages of regression significantly above that caused by these two factors can be considered as due to the slow neutron-boron process in the experiments which combine the irradiation and the localization of the boron or lithium compounds within or around the tumor. A study of the data of experiments 4, 5, 6 and 7 of the table clearly indicates that the regression in mice receiving both irradiation and the boron or lithium injections is considerably higher than in those receiving the injections alone, the percentage differences in these four cases being respectively 16.6, 22.2, 45.0 and 15.5. The differences or boron-neutron cures are not large, but since in all four experiments they are consistent, their significance seems conclusive.

*Discussion.*—The actual variation in the magnitude of these regression differences seems to be subject to any of several explanations: (1) Although the animals, in relation to the source of the irradiation, were placed in such a position so as nearly as possible to equalize the dosage of all the animals being irradiated at one time, complete uniformity was impossible to achieve, due to the architecture of the cyclotron. (2) Fluctuations in the output of the cyclotron during the excessively long radiation periods. (3) Variations in the effectiveness of the injections: the amount of injury, and diffusion factors of the injected materials. (4) Variation in the normal frequency of regression.

The ionization at the site of the injection was not known. As cited above from Sugiura's experiments, a radiation equivalent to 1000 r of x-rays is necessary to cause a 50% regression *in vivo*. With 750 roentgens of x-rays Sugiura reports a 20% regression *in vivo*. Considering the magnitude of our regressions as between 15.5 and 45.0 per cent, and postulating the actual destruction of the malignant cells, it is necessary to assume that an ionization approximately equivalent to 750–1000 roentgens of x-rays was being achieved within or at the tumor, whereas the whole body was being subjected to less than the equivalent of 400 r. We assume that this energy

difference was the result of the ionization caused by the disintegration products of the slow neutron-boron process.

On the other hand, due to the peculiar selective permeability properties of the living cell membrane, we find it difficult to believe, following the injection of boric acid or lithium meta-borate into and around the tumor mass, that as the material slowly goes into aqueous solution a high concentration is maintained uniformly both within and out of the malignant cells. Even if material were injected and retained at the site of the tumor, it was early doubted whether the boric acid or lithium hydroxide would diffuse uniformly throughout the tissue mass in concentrations necessary to be effective in capturing a large proportion of the slow neutrons. Indeed, the question of whether the ions would easily permeate the membranes of tumor cells has not yet been established, and there is little evidence to indicate that they would concentrate in sufficiently large quantities to be effective. It is more likely that they diffuse into and through the intercellular spaces. But in the case of a compact tumor such as Mouse Sarcoma 180 the amount of intercellular diffusion is limited.

For this reason, and because of the relatively high incidence of ulceration around the tumor following the injection-radiation treatment, at the present time the curative regression is interpreted as due to one or a combination of the following factors: (1) alpha particle or proton destruction of some or all of the malignant cells, (2) impairment of the vascular system feeding and draining the tumor, (3) radiation effect on the connective tissue base of the tumor.<sup>6</sup>

One of the interesting aspects of this work is that, unlike x-ray therapy where shielding of tissues not under treatment must be carefully applied, we were able to subject the whole body to the same extrinsic energy as the tumor; but that because of the neutron-boron process we were able to set up a high ionization differential between the tumor and the rest of the body.

The authors consider that for any possible future employment of the slow neutron-boron process in tumor therapy, some device other than simple hypodermic injection should be developed for localizing either boron or lithium or related materials in malignant tissue. This is particularly essential in the case of involved metastasizing and deeply situated growths. Experiments involving the intravenous injection of large particle colloidal dyes to which the lithium atom is attached have indicated considerable localization of lithium in spontaneous mouse tumors. This work is being continued.

It is to be mentioned also that concurrently with the work described in this communication, experiments were undertaken in which boron in one form or another was injected into the mouse testis, followed by slow neutron irradiation. A clearly observable effect on the germinal cells of the seminiferous tubules much more extensive than either the radiation effect alone

or the effect of the chemicals also was observed. These results will be published elsewhere.

*Summary.*—Transplantable mouse sarcomas were injected with various forms of slow-neutron-capturing materials. When the whole animal whose tumor was so injected was irradiated with slow neutrons, a significant increase in tumor regression was observed. This increase is attributed to the localized ionization resulting from the nuclear disintegration products of the capture process.

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<sup>2</sup> The Haskins Laboratories, New York City.

<sup>3</sup> Columbia University. Aid of the Research Corporation is gratefully acknowledged. We wish to express our appreciation to Dr. C. P. Rhoads and Dr. G. Failla for courtesies extended to us at Memorial Hospital, New York City.

<sup>4</sup> The cross-section is a measure of the probability of interaction of the neutron with the nucleus, i.e., if there is a flux of one neutron per centimeter squared per second and one atom per cubic centimeter, the cross-section represents the probability of this neutron being captured by the atom.

<sup>5</sup> Other elements in tissue also capture some slow neutrons but the resulting contribution to *local* ionization is not extensive. It should be noted that the small absorption within the tissue of the 2.2 MEV  $\gamma$ -radiation resulting from slow neutron capture by hydrogen largely offsets the efficiency of this capture process, at least as regards local ionization.

<sup>6</sup> Sugiura (5) and others have demonstrated that the incidence of tumor-takes is significantly lower in areas which have been previously subjected to irradiation, thus indicating the importance of the physiological condition of the tissue base to the proper growth of introduced cancerous tissue.



# GALACTIC AND EXTRAGALACTIC STUDIES, X. NOTE ON LATITUDE EFFECT AND RADIAL GRADIENTS IN THE NORTHERN GALACTIC HEMISPHERE

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Communicated September 10, 1940

1. The preceding paper<sup>1</sup> of this series summarized the magnitudes of some 22,000 galaxies in a five-degree zone encircling the sky at declination  $+43^{\circ}.6$ . The material is suitable for an examination of the dependence of nebular density on galactic latitude, and for the study, through the relative frequency of magnitudes, of radial density gradients at different places along the zone. From a recent survey in the south galactic polar cap,<sup>2</sup> the latitude effect, if present at all, was found to be smaller than other transverse gradients in that region. In an earlier study<sup>3</sup> of "sample regions" in both hemispheres, in which low as well as high latitudes were involved, we found, in essential agreement with Mount Wilson results, the well-known avoidance-zone effect up to latitudes  $\pm 30^{\circ}$ , but in the northern galactic hemisphere no appreciable latitude effect from  $\beta = +30^{\circ}$  to the galactic pole.

TABLE 1  
DISTRIBUTION CONSTANTS FOR 18 PLATES

PLATE	$\beta$	$b$	$m_1$	PLATE	$\beta$	$b$	$m_1$
MC 29493	$+35^{\circ}.5$	0.70	15.58	MC 30038	$+73^{\circ}.6$	0.77	15.30
29215	39.8	0.57	15.16	30129	70.2	0.69	15.37
27687	46.9	0.56	15.13	29962	65.4	0.73	15.60
30078	52.2	0.62	15.18	29979	60.8	0.53	15.05
29265	58.3	0.70	15.33	28949	56.2	0.48	14.82
29283	63.8	0.68	15.30	30079	49.2	0.64	14.91
28788	68.3	0.76:	15.68:	30186	42.7	0.59	14.85
27639	72.1	0.65	15.03	29537	37.5	0.56:	15.02:
27704	74.0	0.64	15.29	30123	31.6	0.62	15.28

2. In the present discussion earlier results are corroborated. For latitudes greater than  $+30^{\circ}$  the irregularities in distribution from place to place (figures 2 and 3) are sufficient to conceal whatever progressive increase there may be in numbers of galaxies with latitude, such as should result from a uniform or approximately uniform layer of absorbing material along the galactic plane. We find also that for the whole area involved the average number of galaxies at each magnitude down to 17.5 differs but little in high latitudes from the average found in the southern galactic hemisphere (equations (1) and (2)). But the space density increases conspicu-

ously with distance for the section of the zone between  $10^h$  and  $14^h$  in right ascension (table 2).

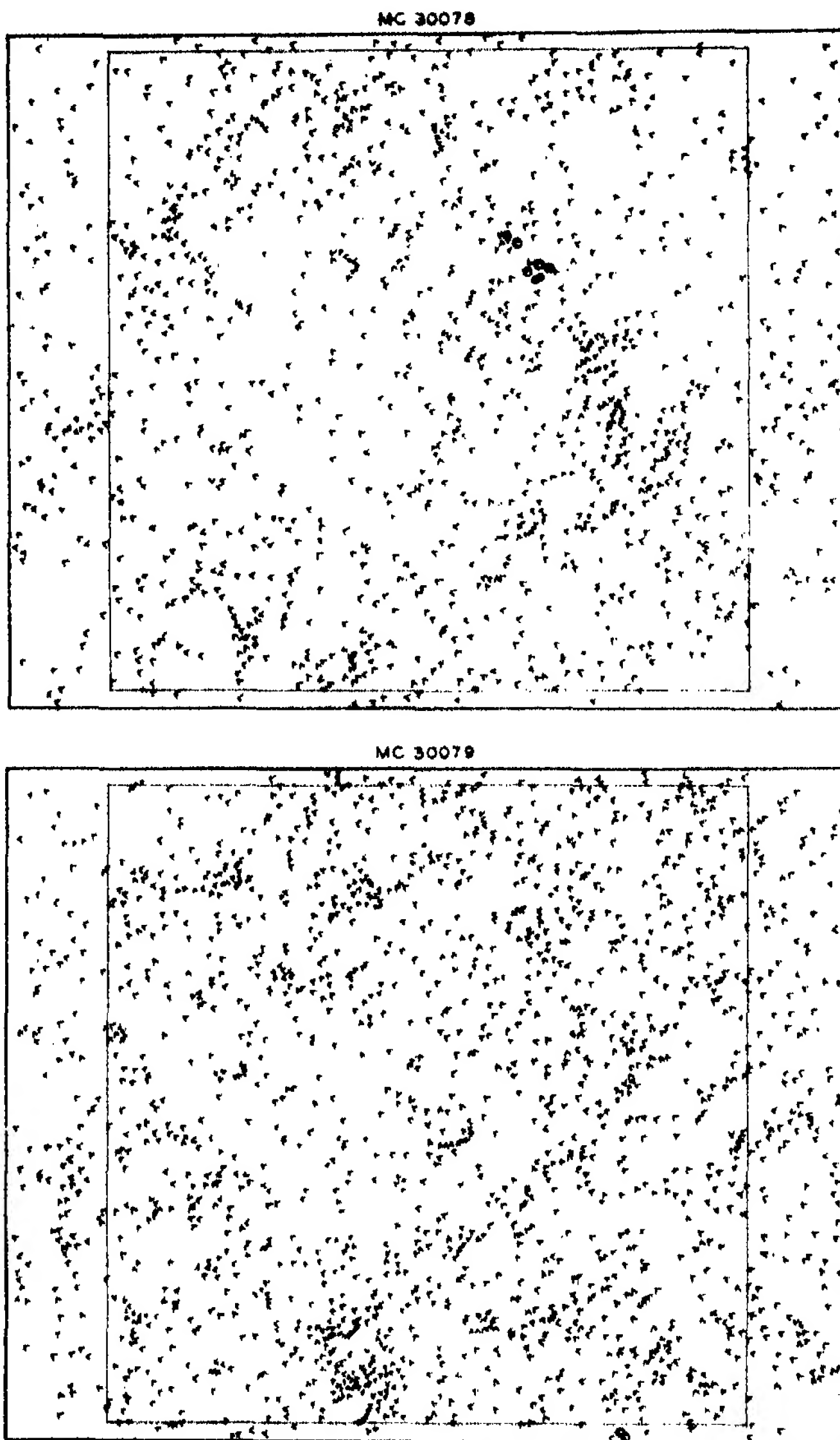


FIGURE 1

Nebular distribution on two plates. Inner areas cover twenty-five square degrees.

The surface distribution is illustrated in figure 1 for two average plates. Stars are not indicated; the positions of NGC objects are circled. The



nebular groupings shown on these plates have not heretofore been recognized as clusters of galaxies.

3. Figure 2 shows the nebular distribution around the zone. For each plate the number of objects per square degree to magnitude 17.5, within the central twenty-five square degrees, is indicated by the ordinates; both right ascension and galactic latitude are used as abscissae. The twenty-five square degrees of each plate is treated as a unit, both in this diagram and in the following discussion. We can see from the figure that within ten degrees of the galactic circle there are practically no galaxies at either of the two crossings of the Milky Way. The relation of nebular density to galac-

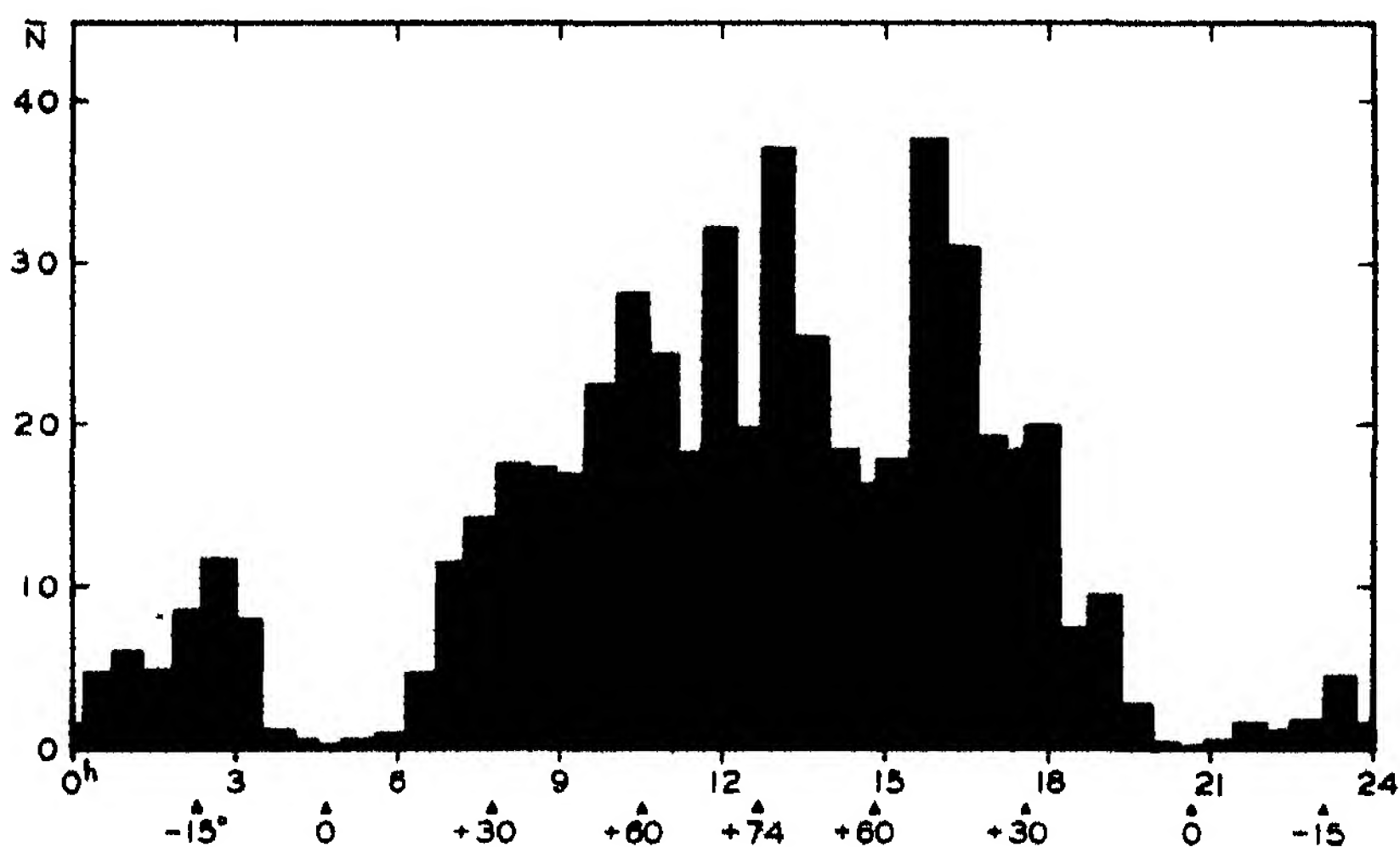


FIGURE 2

Distribution of galaxies to magnitude 17.5 along the five-degree zone. Abscissae are both hours of right ascension and degrees of galactic latitude; ordinates are numbers of galaxies per square degree.

tic latitude is further illustrated in figure 3, where the logarithm of the average number of galaxies per square degree is plotted against galactic latitude for three magnitude limits.

4. If we consider the space absorption and its unevenness as unimportant for latitudes higher than  $\pm 30^\circ$ , we can use this homogeneous material, after correcting for the red-shift, for a study of the nebular distribution parameters,  $b$  and  $m_1$ , in the relation  $\log \bar{N}_m = b(m - m_1)$ . From plots giving for each plate the apparent photographic magnitude,  $m$ , against the logarithm of the number of objects equal to or brighter than that magnitude,  $\log N_m$ , the values of  $b$  and  $m_1$  have been determined graphically for the eighteen plates with  $\beta > 30^\circ$ ; they are given in table 1. These values

are derived on the assumption that the surveys are complete to magnitude 17.5; for the four plates (table 1 in the ninth paper<sup>1</sup>) for which the limits of completeness,  $m_n$ , are a little brighter than magnitude 17.5, appropriate corrections (through the uniform density assumption) have been made. The values of  $b$ , the radial density gradient, vary from 0.48 to 0.77, and in the mean  $\bar{b} = 0.64 \pm 0.02$  (m. e.); the values of  $m_1$ , the space density parameter, vary from 14.82 to 15.68, with the mean  $\bar{m}_1 = 15.22 \pm 0.06$  (m. e.).

If we plot the logarithms of the sums for all eighteen plates (four hundred fifty square degrees) against the apparent magnitudes, we have the result

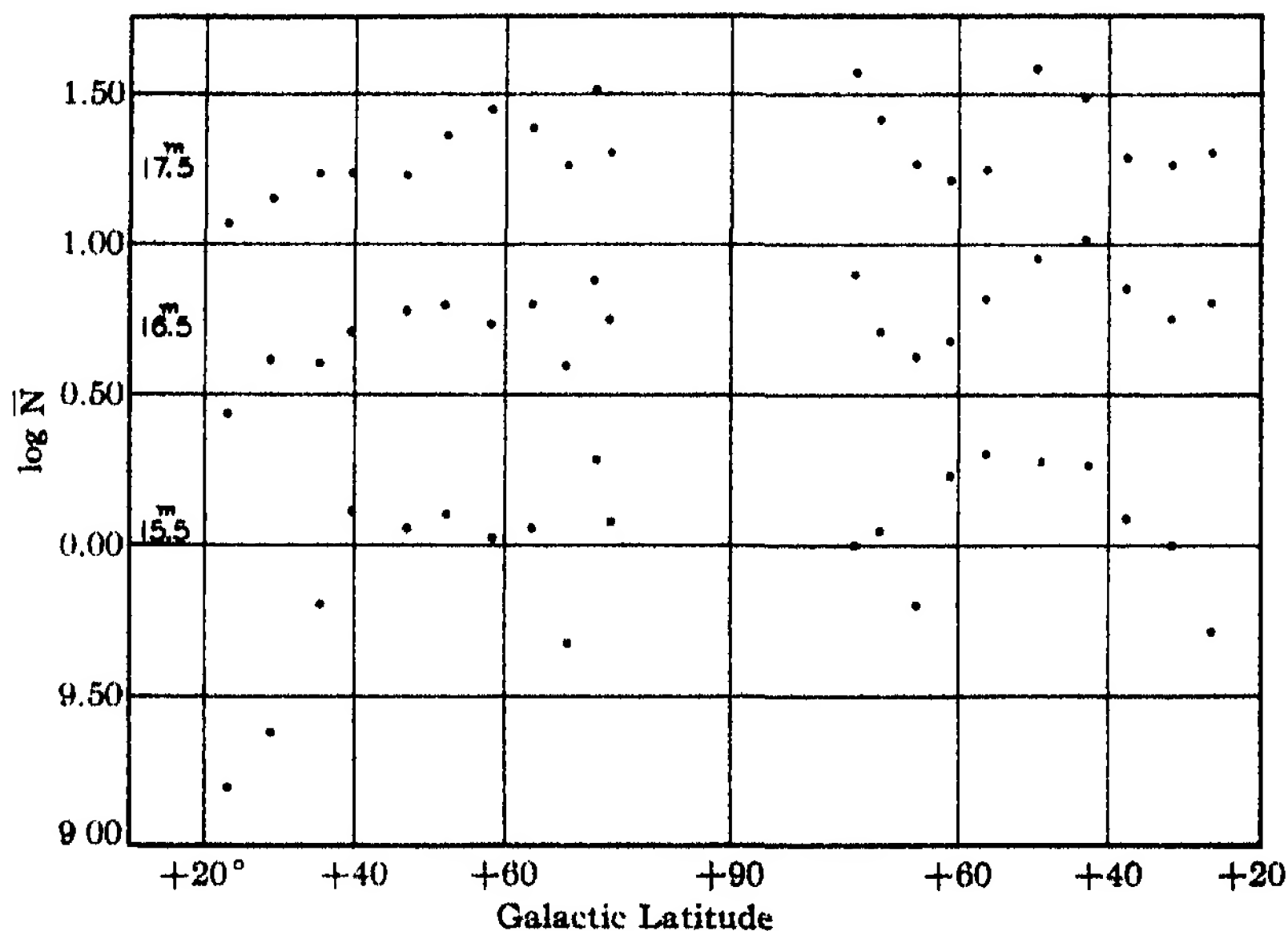


FIGURE 3

Variation of the logarithm of the number of galaxies per square degree with galactic latitude for three magnitude limits. All plates with galactic latitude greater than  $\pm 20^\circ$  are included.

shown in figure 4. The best straight line representation, graphically determined, gives  $b = 0.62$  and  $m_1 = 15.20$ ; a least-squares solution, in which the successive cumulative totals are weighted according to the number of galaxies involved, gives the values that we shall adopt as mean for the zone:

$$\begin{aligned} b &= 0.625 \pm 0.017 \text{ (m. e.)} \\ m_1 &= 15.18 \pm 0.04 \end{aligned} \quad (1)$$

These values should be compared with those obtained for the 198 square

degrees in the south galactic cap,<sup>2</sup> in a survey that extends to somewhat fainter galaxies:

$$\begin{aligned} b &= 0.592 \pm 0.009 (\text{m. e.}) \\ m_1 &= 15.16 \pm 0.02 \end{aligned} \quad (2)$$

The similarity in the values of  $m_1$  in equations (1) and (2) can be taken either as a measure of the essential equality in density in regions that are separated by something like fifty megaparsecs, with the implication of equality of space absorption in the northern and southern directions; or that greater absorption on one side is compensated by low density on the other. The difference between the values of  $b$ , 0.033, is not much larger than its mean error, but the steeper gradient on the north may be significant in the local structure of the metagalaxy.

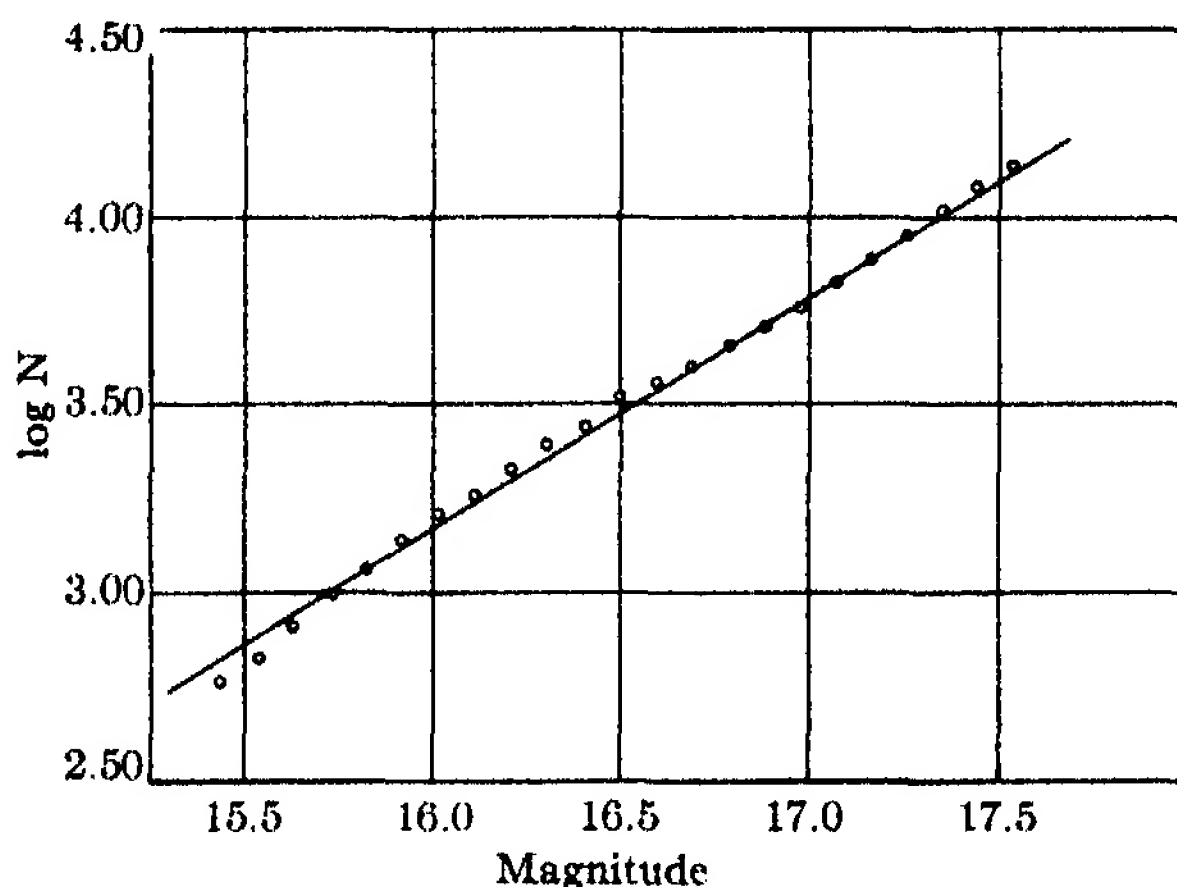


FIGURE 4

Frequency of magnitudes, corrected for red-shift, for the eighteen plates of highest galactic latitude (450 square degrees). Abscissae are photographic magnitudes; ordinates are logarithms of cumulative total numbers of galaxies.

The close similarity of average density at magnitude 17.5 in the two hemispheres may be quite accidental and disappear altogether when larger areas of the high latitude sky are surveyed. In fact, the values of  $b$  and  $m_1$  vary considerably from plate to plate along the  $5^\circ$  zone, as shown in table 1. In table 2 we give results of determinations by least squares of the parameters when the material from the eighteen plates is divided into early and late right ascension groups, as well as when the nine plates of highest galactic latitude are treated as a group. Practically the same results would be obtained if the means of the graphically determined values in table 1 were computed for the same groups of plates.

TABLE 2  
MEAN DISTRIBUTION CONSTANTS

RIGHT ASCENSION INTERVAL	NUMBER OF PLATES	$b$	$m_1$
$8^h15^m0 - 12^h27^m6$	9	$0.646 \pm 0.015(\text{m. e.})$	$15.27 \pm 0.03(\text{m. e.})$
13 04.1 – 17 26.6	9	$0.627 \pm 0.020$	$15.12 \pm 0.04$
10 22.1 – 14 42.8	9	$0.682 \pm 0.010$	$15.32 \pm 0.02$
8 15.0 – 17 26.6	18	$0.625 \pm 0.017$	$15.18 \pm 0.04$

For the group of nine plates of highest latitude (225 square degrees containing 5504 galaxies of magnitude 17.5 and brighter) the space density,  $m_1 = 15.32$ , is the lowest. The gradient,  $b = 0.68$ , is much steeper than for the other combinations—possibly an effect of unrecognized clusters or clouds of galaxies that begin to appear near the fainter magnitude limit on some of the plates. The full picture of local metagalactic structure in these northern regions must await considerable further work on nebular magnitudes.

<sup>1</sup> These PROCEEDINGS [ninth paper of the series, Sept., 1940].

<sup>2</sup> Op. cit., 26, 166–176 (1940).

<sup>3</sup> Op. cit., 19, 389–393 (1933); *Harv. Bull.* 894, 10 (1934).

## THE EFFECT OF SHEAR INSTABILITY ON THE TRANSVERSE CIRCULATION IN THE ATMOSPHERE

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The motion of the atmosphere can be considered as a mean flow which is of very large scale and is only slowly changed and, superimposed on this the low level, smaller scale phenomena usually associated with the polar front. If the mean pressures over a period of about a week are plotted, it is seen that the latter disturbances are averaged out, and only the large-scale mean motion is shown. Such a plot of the Northern Hemisphere shows, in addition to the mean westerly flow of air, large-scale closed isobaric systems spaced at comparatively regular distances on the surface of the earth. These include the Aleutian and Icelandic low-pressure areas to the north of the westerlies and the Pacific and Bermuda high-pressure areas to the south of the westerlies. The maximum number of high-pressure cells seems to be about six.

As the position and strength of these disturbances apparently control the mean path of the low-level storms, a knowledge of the properties of these systems would appear extremely desirable for any long-range weather

forecasting. One aspect of this problem has been investigated by Rossby<sup>1</sup> who showed that wave disturbances of about the observed wave-length can exist as a result of the variation of the Coriolis acceleration with latitude. In the present paper, the results of an investigation of another aspect of the problem arising from the existence of the shearing motion on either side of the belt of westerlies will be given. This investigation was started as a result of conversations with Th. von Kármán and C. G. Rossby and was carried out in coöperation with the United States Department of Agriculture under the provisions of the Bankhead-Jones Act.



FIGURE 1

In this investigation it was assumed that the atmosphere could be treated as a single layer of fluid of constant density with the vertical velocities being of small importance so that the pressure was determined by the hydrostatic law. In addition the variation of the Coriolis parameter with latitude and the effects of friction were neglected.

The notation used in the discussion is as follows:

- $x, y$  = Cartesian coördinates on a rotating disc
- $r, \theta$  = Polar coördinates on a rotating disc
- $u$  = velocity in  $x$  direction
- $v$  = velocity in  $y$  direction
- $\omega$  = angular velocity of disc
- $h$  = depth of fluid
- $g$  = acceleration due to gravity
- $K_0(x)$  = modified Bessel function of the second kind.

If the motion could have started from rest with a uniform depth,  $h_0$ , the principle of conservation of absolute vorticity states that

$$\frac{\partial v}{\partial x} - \frac{\partial u}{\partial y} = \frac{2\omega}{h_0} (h - h_0). \quad (1)$$

If the velocity components are eliminated from this equation by means of the geostrophic wind equations,

$$-2\omega v = -g \frac{\partial h}{\partial x} \quad (2)$$

and

$$2\omega u = -g \frac{\partial h}{\partial y},$$

an expression determining the depth of the atmosphere (i.e., sea-level pressure) is obtained. This is

$$\frac{\partial^2 h}{\partial x^2} + \frac{\partial^2 h}{\partial y^2} - \frac{4\omega^2}{gh_0} (h - h_0) = 0. \quad (3)$$

The only steady state solution of this equation which vanishes at infinity and which represents flow in circles about the origin is

$$\frac{h - h_0}{h_0} = AK_0 \left( \frac{2\omega r}{\sqrt{gh_0}} \right) \quad (4)$$

where  $A$  is an arbitrary constant. If  $A$  is positive, the motion is anticyclonic; if  $A$  is negative, the motion is cyclonic. All the motions considered in this investigation were produced by superposition of vortices of these types.

The shear field south of the westerlies can be considered to a first approximation as a sheet of vorticity separating a moving current from a stationary one. Such a vortex sheet was shown to be dynamically unstable. A similar calculation by Pekeris yielded the same result. This means that the shear fields bordering the westerlies tend to break up into discrete eddies. If there should be stable formations of vortices, dynamic forces would cause these small eddies to collect into one of the stable formations. An investigation of the stable vortex formations should thus give a clue to possible patterns of motion.

For small enough distances from the vortex given in equation (4) or, more exactly, if  $\frac{2\omega r}{\sqrt{gh_0}} \ll 1$ , the velocity varies inversely with the radius just as in an ordinary two-dimensional vortex in an incompressible fluid without Coriolis forces, and similar results regarding stability will be obtained provided the vortex spacing is such that the above-mentioned inequality is satisfied; however, the geostrophic wind equations, equation (2), are greatly in error in this region so the results of investigations with ordinary vortices cannot be applied directly.

A single infinite row of vortices as in figure 1 is readily seen to be in equilibrium, but investigation proves the equilibrium to be unstable, and this type of pattern could not be expected to form.

As there is a shear field on either side of the westerlies, a double row of vortices is a better fit to this shear field. There are two possible equilibrium arrangements for such a double-row system as shown in figure 2. Such systems are similar to the Kármán "vortex street" and were investigated in a similar manner.<sup>2</sup> The results of this calculation show that the symmetrical arrangement is unstable while the asymmetrical arrange-



ment is stable for a certain range of values of  $d/l$  which depends on the value of  $\frac{2\omega l}{\sqrt{gh_0}}$  as shown in the following table:

STABLE RANGE OF VALUES OF  $d/l$  FOR VORTEX STREET

$\frac{2\omega l}{\sqrt{gh_0}}$	UPPER LIMIT	LOWER LIMIT
0	0.281	0.281
1	0.300	0.281
2	0.350	0.280

This result is shown graphically in figure 3. It should be noted that for small values of  $\frac{2\omega l}{\sqrt{gh_0}}$  the result,  $d/l = 0.281$ , is exactly that obtained by Kármán. The flow pattern associated with the vortex street shows a jet of westerlies with the vortices drifting to the east.

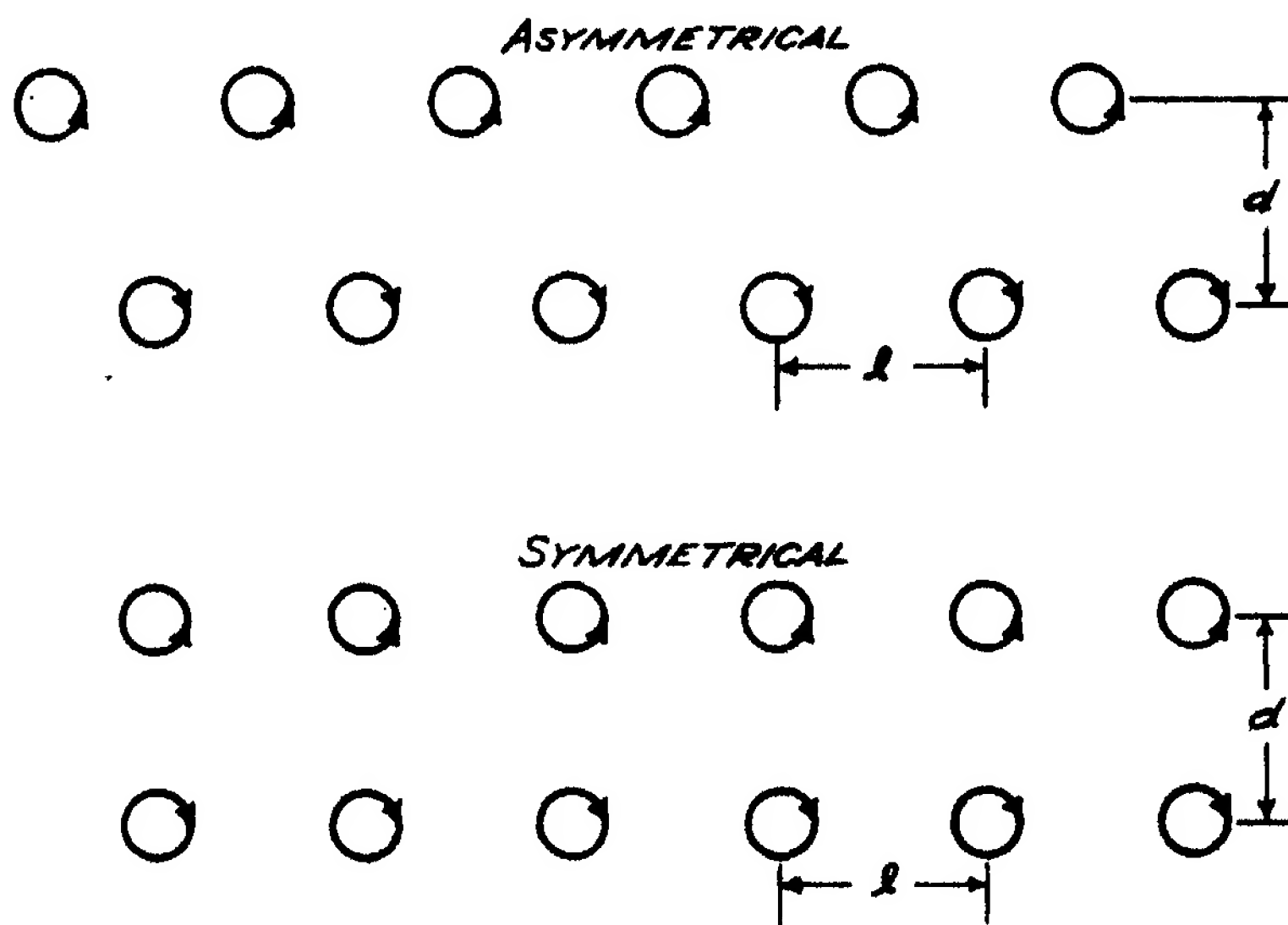


FIGURE 2

These results are directly applicable to the spherical earth only when the distances  $l$  and  $d$  are small compared with the earth's radius. This is equivalent to saying that the general circulation is very weak.

The observed pressure systems have spacings of the order of the earth's radius, and it is necessary to consider the fact that the path of the westerlies is actually curved to investigate motions on this scale. A general solution of the problem of the stability of double-ring systems is very difficult, but the nature of the results can be estimated by considering some limiting

cases. The preceding calculation gives a limiting case where the circulation is very weak. Normally the shear field to the north of the westerlies is weaker than that to the south. If the northern shear can be entirely neglected, the only possible steady pattern consists of equal anticyclones on the corners of a regular polygon and of a constant distance,  $a$ , from the north pole as in figure 4. Investigation shows that such a system is stable for two, three, four, five or six vortices. For seven vortices the arrangement is stable if  $(2\omega a/\sqrt{gh_0}) > 71$ . For more than seven vortices, the arrangement is unstable. For small values of  $2\omega a/\sqrt{gh_0}$  this is in agreement with the work of J. J. Thomson.<sup>3</sup> As values of  $2\omega a/\sqrt{gh_0}$

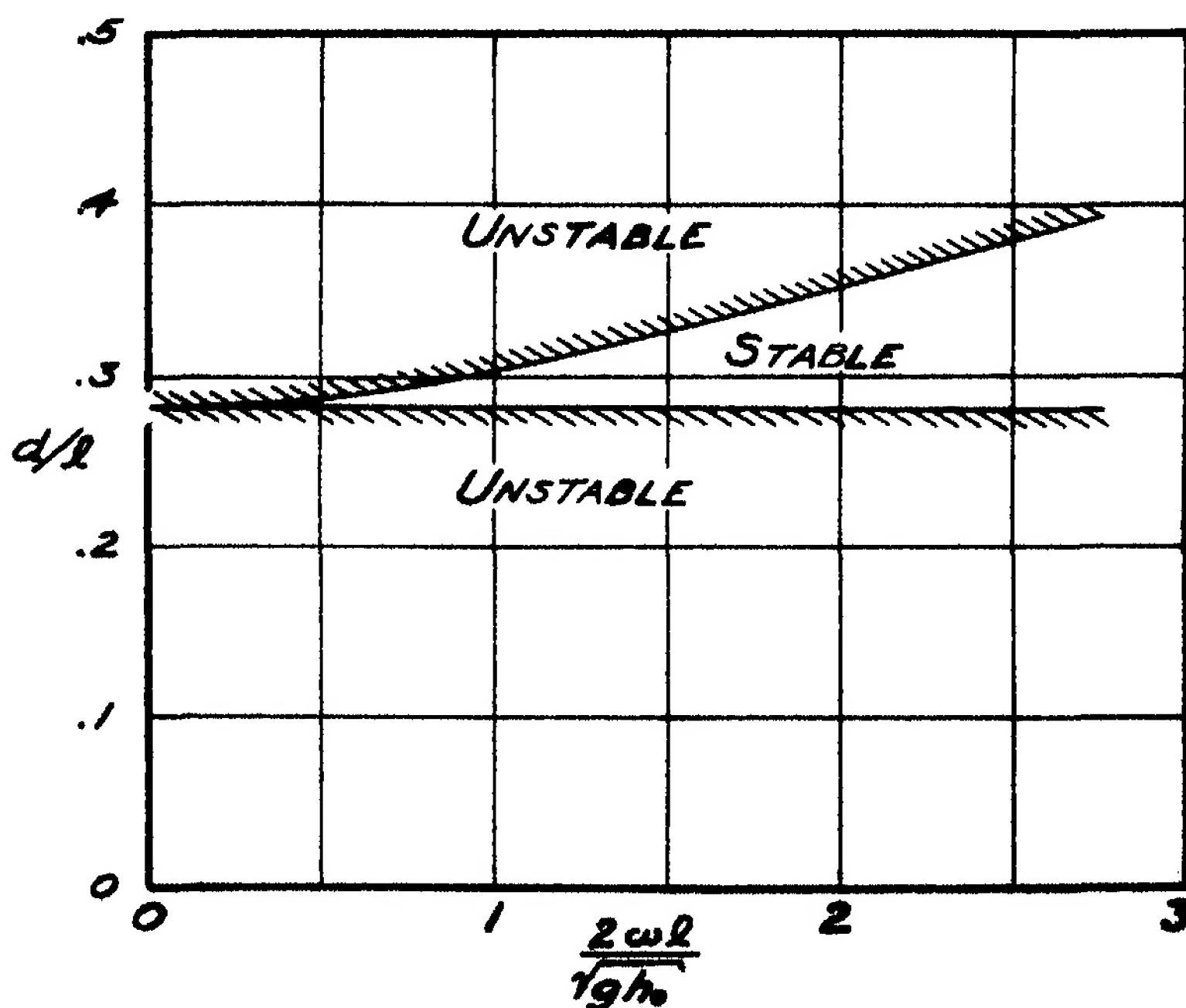


FIGURE 3

greater than 71 are of no apparent significance, these results may be summarized by saying that a ring of six or less vortices is stable. The flow pattern associated with a ring of anticyclones shows a wide jet of westerlies with the anticyclones drifting westward.

The effect of the shear field north of the westerlies may be roughly estimated by placing a single fixed cyclonic vortex at the pole as in figure 5. Such a vortex pattern gives the same results as the single ring provided the cyclone is weak enough; however, as the polar cyclone becomes stronger, the maximum number of anticyclones decreases. If the polar cyclone is strong enough to halt the westward movement of the anti-

cyclones, the system is unstable even with only two anticyclones. This configuration shows a wide band of westerlies and corresponds to a state of strong general circulation. This should correspond quite closely to the observed motion in the atmosphere as the shear field north of the westerlies is weaker than that to the south. As the polar air mass tends to rotate

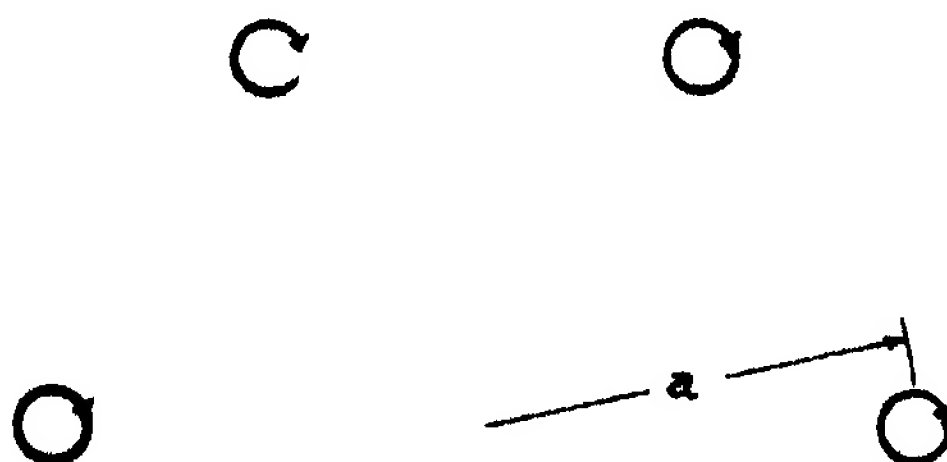


FIGURE 4

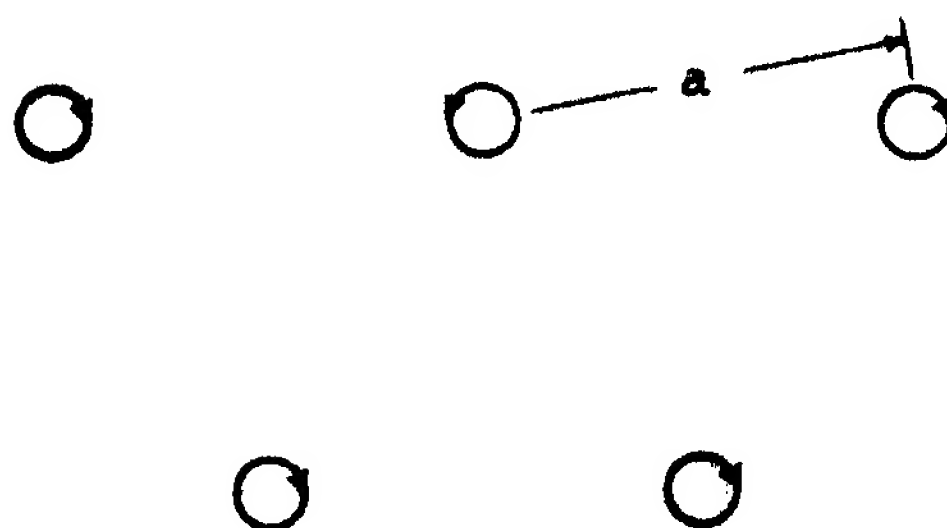


FIGURE 5

as a whole, an increase in the westerly winds, on a percentage basis, increases the shear to the north of the westerlies faster than that to the south. Thus a strong atmospheric circulation corresponds to a large value of the ratio of the vorticity north of the westerlies to the vorticity south of the westerlies, and a weak atmospheric circulation corresponds to a small value of the ratio. From the preceding results, this implies that with

strong circulation only two or three high-pressure cells would be found, whereas with weak circulation stable forms having up to six cells could be found.

As a result of these calculations it is evident that the shear fields next to the westerlies will roll up and form a series of closed isobaric systems with high-pressure cells to the south of the westerlies and low-pressure cells to the north of the westerlies. It appears that a maximum of six high-pressure cells will be observed although more are possible if the belt of westerlies were to become extremely narrow. The low-pressure cells will be asymmetrically placed with respect to the high-pressure cells as in figure 2. Of the two limiting cases, one progressed westward and the other eastward, so one might expect the intermediate systems to be practically stationary. This is in rough agreement with the idea that the position of such systems ought primarily to be determined by thermodynamic considerations, so that the stable vortex configurations found in the atmosphere might be expected to be those that are stationary. It further appears that only two or three high-pressure cells will be found with the strongest atmospheric circulation with the maximum number of cells increasing as the strength of the circulation diminishes, and one might expect a strong inverse correlation between the strength of the circulation and the number of high-pressure cells. This result is well verified by Northern Hemisphere mean-pressure charts although no attempt has been made to correlate these factors in a quantitative fashion.

<sup>1</sup> C. G. Rossby, "Relation Between Variations in the Intensity of the Zonal Circulation of the Atmosphere and the Displacements of the Semi-Permanent Centers of Action," *Jour. Marine Research*, 2, No. 1, 38 (1939).

<sup>2</sup> W. F. Durand, *Aerodynamic Theory*, 2, p. 342.

<sup>3</sup> J. J. Thomson, *Motion of Vortex Rings*, p. 94.

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## ON THE MORTALITY IN HUSBANDS AND WIVES

BY ANTONIO CIOCCO

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The study of morbidity and mortality in husbands and wives when properly controlled by other pertinent data offers the possibility of throwing some light regarding the action of a number of factors, environmental as well as hereditary, on the diseases about which so little is known and that are so prevalent in adult and old age. This consideration has served as a stimulus for the initiation of the study reported in this note.\*

The material for this study has been obtained from the death records of

1898 to 1938 filed in the Washington County (Maryland) Health Department. The procedure of collecting the necessary data was as follows:

1. Transcripts were made of all the death records concerning white persons stated to have been (a) married and (b) widowed at the time of death.
2. The records for the married and for the widowed persons, respectively, were alphabetized and divided according to sex.
3. The records of married males have been matched with those of widowed females and the records of widowed males with those of married females.

To date the records of 2578 couples, both of whom are dead, have been collected and the information verified. This number constitutes 45 per cent of all the couples that it could have been at all possible to discover by the means employed.

Among the 2578 couples, the husband and wife of 7 died simultaneously or within 24 hours from homicide-suicide, accidents, etc. They have been excluded from further consideration and so the material on which this study is based is constituted by the death records of 2571 couples.

*Correlation in the Age at Death of Husbands and Wives.*—The product-moment coefficient of correlation between the length of life in husbands and in wives is found to equal  $+0.5594 \pm 0.0092$ . The tendency for marital partners to have the same duration of life is apparently independent of whether the husband and wife died of the same or of different causes. When only the couples who died from different causes are considered the correlation coefficient  $r = +0.5485 \pm 0.0103$  which is obviously only slightly inferior to the value observed for all couples. The correlation coefficient calculated for the age at death of husbands and wives who died from the same cause is  $+0.6454 \pm 0.0202$  which is higher than that observed for all couples. It would seem then that the correlation in length of life of husbands and wives is not due to whatever marital association there may be relative to the cause of death. On the other hand, when husbands and wives do die from the same condition they demonstrate also a greater tendency to the same life span.

*Causes of Death in Husbands and Wives.*—The cause of death stated on the death certificate has been coded according to the International List (1929 rev.); and when two or more causes were mentioned, the rules given in the third edition of the Manual of Joint Causes of Death (1933) have been followed.

The frequency with which the stated causes of death occurred in the 2571 married couples is shown in table 1.

In table 1 are given the observed number of husbands and wives who died from stated causes and the number expected assuming random matings according to cause of death. When the observed and the expected numbers are compared for all of the combinations in table 1 and the chi-square

TABLE 1  
OBSERVED AND EXPECTED (IN PARENTHESIS) NUMBER OF HUSBANDS AND WIVES WHO DIED FROM STATED CAUSES

CAUSE OF DEATH OF HUSBAND	CAUSE OF DEATH OF WIFE								ALL CAUSES
	TUBERCULOSIS (ALL FORMS)	ARTERIO- SCLEROSIS	INFLUENZA AND PNEUMONIA (ALL FORMS)	CANCER AND OTHER MALIGNANT TUMORS	NEPHRITIS	CEREBRAL HEMORRHAGE	HEART DISEASES	OTHER AND ILL- DEFINED CAUSES	
Tuberculosis (all forms)	20 (7.4)	4 (4.5)	11 (10.1)	8 (12.5)	7 (10.6)	13 (14.9)	29 (31.0)	25 (26.0)	117
Arteriosclerosis	10 (8.9)	6 (5.3)	14 (12.8)	10 (14.9)	16 (12.7)	19 (17.9)	32 (37.1)	33 (31.2)	140
Influenza and pneumonia (all forms)	12 (11.4)	9 (6.9)	27 (15.5)	15 (19.2)	16 (16.3)	13 (23.9)	40 (47.7)	48 (40.1)	180
Cancer and other malig- nant tumors	9 (11.9)	5 (7.1)	10 (16.1)	29 (19.9)	14 (16.9)	25 (23.9)	64 (49.5)	31 (41.6)	187
Nephritis	15 (19.0)	12 (11.4)	30 (25.8)	25 (31.9)	33 (27.1)	36 (38.2)	77 (79.2)	71 (66.5)	299
Cerebral hemorrhage	17 (25.4)	14 (15.2)	23 (34.5)	50 (42.6)	46 (36.2)	56 (51.0)	108 (106.0)	86 (89.0)	400
Heart diseases	39 (38.4)	22 (23.4)	44 (53.0)	66 (65.5)	50 (55.6)	84 (78.4)	184 (162.6)	125 (136.6)	614
Other and ill-defined causes	41 (40.2)	26 (24.2)	63 (54.7)	71 (67.6)	51 (57.4)	82 (80.9)	147 (167.9)	153 (141.0)	634
All causes	163	98	222	274	233	328	681	572	2571



test applied, it is found that the deviation between the observed and the expected amounts to 4.5 times its standard error. Therefore, from a statistical standpoint, it appears that relative to cause of death the husbands and wives of this sample do not constitute a random combination of men and women.

A more exact evaluation of this apparent association between husbands and wives relative to cause of death may be obtained when the data regarding each of the seven specific causes are arranged in a four-fold tabulation and the chi-square is calculated for each four-fold table. It can then be shown that the association between husband and wife with respect to tuberculosis lies far beyond the limits of chance deviation ( $P = < 0.0001$ ). The frequency with which both husbands and wives have died of influenza and pneumonia is also significantly larger than expected on the basis of random assortment ( $P = 0.0016$ ). With regard to the concurrence of deaths of husbands and wives from cancer and from heart diseases, respectively,  $P$  approximates 0.025 and the deviations from chance expectancy may also be regarded as statistically significant. Instead, for nephritis, cerebral hemorrhage and arteriosclerosis, respectively, the concurrence in husbands and wives does not deviate significantly from what would be expected assuming random mating. In sum, these data reveal that when a husband or wife died of tuberculosis the relative number of their spouses who also died from this cause is three times as high as that of the spouses of persons who died from other causes. Influenza and pneumonia as a cause of death occur almost twice more often among the husbands and wives of individuals who died from this cause than among the deceased spouses of those who did not. This ratio is 1.5 in the case of cancer and 1.2 in the case of heart diseases.

To determine whether age and the time and order of death of the two spouses could affect the frequency with which both died from the same cause, a supplementary control sample to be used in lieu of the calculated expected was formed according to the following procedure:

1. All the records concerning husbands whose death preceded that of the wives were alphabetized and the same was done for the records of wives whose death preceded that of the husbands.

2. For each husband whose death preceded that of the wife, a widow was sought in the files of unmatched widows (already alphabetized as it will be recalled) having the following characteristics: (a) the age at death within  $\pm 5$  years that of the wife, (b) time of death within  $\pm 5$  years of that of the wife. The first widow in alphabetical order who had these characteristics was selected as a control.

3. For each wife whose death preceded that of the husband a control widower was selected in the same manner as described in 2.

Among the control couples thus obtained, the number found in which

both the male and female died from (a) tuberculosis, (b) influenza and pneumonia, (c) cancer and (d) heart diseases are shown in table 2 and compared to the number of marital couples both of whom have died from one of these causes and to the number expected assuming random mating.

TABLE 2

NUMBER OF COUPLES IN WHICH THE MAN AND WOMAN BOTH DIED FROM THE SAME CAUSE. *A* = OBSERVED NUMBER OF HUSBANDS AND WIVES. *B* = EXPECTED NUMBER OF HUSBANDS AND WIVES ASSUMING RANDOM ASSORTMENT OF MEN AND WOMEN. *C* = NUMBER OF CONTROL, NON-MARITAL, COUPLES

CAUSE OF DEATH	<i>A</i>	<i>B</i>	<i>C</i>
Tuberculosis (all forms)	20	7.4	9
Influenza and pneumonia (all forms)	27	15.5	19
Cancer and other malignant tumors	29	19.9	19
Heart diseases	184	162.6	166

From table 2 it is immediately apparent that the number of control couples and the number of the expected are to all intents and purposes the same. The closeness in the agreement between the control and calculated data is remarkable and shows that what differences there may exist relative to the age and period of death of the spouses do not alter the deviations found between observed and expected, and it also indicates that within the limits of this comparison the calculated numbers actually represent the expectations assuming random heterosexual mating.

*Discussion.*—There are two series of findings resulting from this first analysis of the mortality in husbands and wives. The first concerns the correlation in the length of life of the two marital partners. This correlation is found to be positive and high and its value is only slightly increased when the cause of death is the same in the husband and wife, and it is very little decreased when the cause is different. Since the same specific causation or disease entity (so far as can be learned from the death certificate) is not apparently responsible for the correlated length of life in husbands and wives it would seem appropriate to have recourse to those vague but understandable terms such as resistance or vitality and say that the phenomenon observed indicates the segregation and pairing, through marriage, of individuals having a similar degree of vitality or resistance to fatal pathological processes.

The second series of findings brings out that besides the existence of a high correlation in the length of life of husbands and wives there is a tendency for marital partners to die from the same cause when one of the mates dies from either tuberculosis, influenza and pneumonia, cancer, or heart diseases. For the last three groups of causes such a relationship has never been observed before, at least to the writer's knowledge, although it could have been expected regarding influenza and pneumonia. For the three causes mentioned, particularly for heart diseases, the tendency is not very

marked but is apparently significant from a statistical standpoint. The concentration of tuberculosis deaths among marital partners has long been recognized and students of tuberculosis have always been surprised to find that this association is not higher.

The hypotheses that on the basis of information now available could reasonably be formulated to elucidate the findings of this study may be summed up as follows:

1. The correlation in length of life or general vitality of husbands and wives could be due to conscious or unconscious marital selection although the economic level or economic changes undoubtedly play some part.
2. Tuberculosis is a chronic infectious disease and the association between husbands and wives relative to the mortality from this cause could be thus easily explained. But, in addition, marital selection both from the somatic and social aspects are probably important.
3. In the case of influenza and pneumonia the transmission of the pathogenic agents from one spouse to the other could be assumed to be the factor involved in the association noted. The transmission, however, due to the interval observed between the deaths of the two partners must be indirect; unless it is believed that one partner becomes a carrier. On the other hand, the common environment and the general economic level play a rôle in the incidence of this disease as apparently does also the somatic constitution. It is of interest to point out that the highest association for cause of death in husbands and wives is found relative to tuberculosis and influenza and pneumonia. Both are infectious diseases and for both one can postulate a number of factors that could produce the concurrence.
4. With respect to heart diseases, marital contagion might be an element if the etiological factor in rheumatic heart disease is definitely shown to be infectious, otherwise the somatic and constitutional aspects of marital selection might be considered.
5. None of the factors mentioned appear to explain the association between husbands and wives relative to death from cancer. Yet on logical grounds it would seem that the hypotheses outlined fairly exhaust the possibilities in this respect and consequently one of them must provide the necessary answer.

The complete inability to give an explanation concerning the cancer findings on the basis of present knowledge and the uncertainty regarding the views advanced about the other causes, heart diseases especially, while immediately discouraging, also seem to point the way for further investigations on these as well as other diseases. In particular, these observations serve to delineate more sharply the practical importance of studying the familial aggregate in relation to problems of health and disease.

\* A full report will appear in a forthcoming number of *Human Biology*.

## ON AN INEQUALITY OF GRUNSKY

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Communicated September 6, 1940

1. Suppose that  $W$  is an arbitrary Riemann domain. We let  $n(w)$  be the number of times  $W$  covers the point  $w$ , and define\*

$$p(R) = \frac{1}{2\pi} \int_{-\pi}^{\pi} n(Re^{i\Phi}) d\Phi. \quad (1.1)$$

For simplicity we write  $n_0 = n(0)$ . The purpose of this brief note is to prove the following theorem:

THEOREM 1. Suppose that  $n_0 > 0$ , and that

$$\int_0^{R_1} p(R) d(\pi R^2) \leq n_0 \pi R_1^2 \quad (H)$$

for all  $R_1 > 0$  (mean  $n_0$  - valency). Then

$$\exp \left\{ \frac{1}{n_0} \int_0^{\infty} \lg R^2 d[-p(R)] \right\} \leq \frac{1}{n_0} \int_0^{\infty} R^2 d[-p(R)], \quad (1.2)$$

and there is equality if, and only if, the left-hand side is infinite, or  $p(R) \equiv n_0$  for  $0 \leq R < R_0 < \infty$ , and  $p(R) \equiv 0$  elsewhere.†

Since

$$\frac{1}{n_0} \int_0^{\infty} d[-p(R)] = \left[ -\frac{1}{n_0} p(R) \right]_0^{\infty} = 1,$$

Theorem 1 is a case of the theorem of the arithmetic and geometric means with weight function  $d[-p(R)]$  which, it is easy to see, is not necessarily of constant sign. The inequality (1.2) (with a different formulation) has been proved by Grunsky‡ subject to the stronger hypothesis that  $n(w) \leq n_0 = n(0)$ . My main purpose is to give an extremely simple proof of this result; the generalization (to mean  $n_0$ -valency) follows incidentally. If we write  $w = Re^{i\Phi}$ , and denote by  $B$  the boundary of  $W$ , the inequality (1.2) can be written in the alternative form:

\* Compare Spencer, reference 4 at end of paper.

† " $f \equiv g$ " here means  $f = g$  except in a null set. If we add to Theorem 1 the hypothesis that  $p(R) \equiv n_0$  in a neighborhood of  $R = 0$ , we may state it as a theorem in real variables without reference to Riemann domains. It is possible to develop the theorem further in this direction.

‡ See reference 3 at end of paper.

$$\exp \left\{ \frac{1}{2n_0\pi} \int_B \lg R^2 d\Phi \right\} \leq \frac{1}{2n_0\pi} \int_B R^2 d\Phi,$$

where (for example) we define  $\int_B = \lim \int_{B'}$  as  $B' \rightarrow B$  from the interior of  $W$ . We may also interpret  $\int_B \dots d[-p(R)]$  as  $\lim \int_{B'}$ .

2. We may suppose that

$$\frac{1}{n_0} \int_0^\infty R^2 d[-p(R)] = R_0^2 < \infty; \quad (2.1)$$

otherwise the theorem is true (and trivial). We then define

$$p_0(R) = \begin{cases} n_0, & \text{if } 0 \leq R < R_0, \\ 0, & \text{otherwise.} \end{cases}$$

Since  $W$  covers  $w = 0$  with multiplicity  $n_0$ , there exists a closed circle of radius  $r$  and center  $w = 0$  every point  $P$  of which is covered by  $W$  at least  $n_0$  times. Since, by (H), it is covered on the average at most  $n_0$  times, it follows that the circle is covered exactly  $n_0$  times. Hence  $p(R) = n_0$  for  $0 \leq R \leq r$ , and so

$$\int_0^\infty \lg R^2 d[-p(R)] = \int_r^\infty \lg R^2 d[-p(R)] = [\lg R^2 [-p(R)]]_r^\infty + \int_r^\infty p(R) d[\lg R^2]$$

by a partial integration;

$$= \int_r^\infty p(R) d[\lg R^2] - n_0 \lg \frac{1}{r^2}, \quad (2.2)$$

since  $p(\infty) = 0$ , and  $p(r) = n_0$ . (2.2) may be written in the form

$$\int_r^\infty p(R) \frac{d(R^2)}{R^2} - n_0 \lg \frac{1}{r^2},$$

and a second partial integration now gives

$$\left[ \frac{1}{R_1^2} \int_r^{R_1} p(R) d(R^2) \right]_{R_1=r}^{R_1=\infty} + 2 \int_r^\infty \left\{ \int_r^{R_1} p(R) d(R^2) \right\} \frac{dR_1}{R_1^3} - n_0 \lg \frac{1}{r^2} = 2 \int_r^\infty \left\{ \int_r^{R_1} p(R) d(R^2) \right\} \frac{dR_1}{R_1^3} - n_0 \lg \frac{1}{r^2}. \quad (2.3)$$

Next,

$$\int_r^{R_1} p(R)d(R^2) = \int_0^{R_1} p(R)d(R^2) - \int_0^r p(R)d(R^2) = \int_0^{R_1} p(R)d(R^2) - n_0 r^2 \leq \text{Min} \{n_0 R_1^2, n_0 R_0^2\} - n_0 r^2 = \int_r^{R_1} p_0(R)d(R^2) \quad (2.4)$$

by (H) and (2.1), since

$$\int_0^{R_1} p(R)d(R^2) \leq \int_0^\infty p(R)d(R^2) = \int_0^\infty R^2 d[-p(R)] = n_0 R_0^2.$$

Substituting from (2.4) into (2.3), we obtain that

$$\int_0^\infty \lg R^2 d[-p(R)] \leq \int_0^\infty \lg R^2 d[-p_0(R)], \quad (2.5)$$

and therefore

$$\exp \left\{ \frac{1}{n_0} \int_0^\infty \lg R^2 d[-p(R)] \right\} \leq \exp \left\{ \frac{1}{n_0} \int_0^\infty \lg R^2 d[-p_0(R)] \right\} = \exp \{ \lg R_0^2 \} = R_0^2 = \frac{1}{n_0} \int_0^\infty R^2 d[-p(R)]$$

by (2.1). This proves the theorem, apart from the statement about the case of equality.

But this is easily disposed of. For if  $p(R) \not\equiv p_0(R)$ , then for a set  $E$  of  $R_1$  of positive measure

$$\int_0^{R_1} p(R)d(R^2) < n_0 R_1^2, \quad (2.6)$$

if  $R_1 \in E$ . Since (2.6) plainly implies inequality at (2.5), it therefore implies it at (1.2).

3. I conclude with an application of Theorem 1. Suppose that  $f(z)$ , regular in  $|z| < 1$ , maps the unit circle  $|z| < 1$  on a (Riemann) domain  $W$ , and that  $f(0) = 0$ . Let  $W^*$  be the domain composed of those points of  $W$  which are visible to an eye placed at  $w = 0$  if all boundary elements  $B$  of  $W$  are opaque, and let  $Z^* = f^{-1}(W^*)$ . Then  $Z^*$  and  $W^*$  are schlicht domains which contain the points  $z = 0$  and  $w = 0$ , respectively, and  $W^*$  is the "star" of  $W$  with respect to the point  $w = 0$ . If we write  $V(W^*)$  and  $V(Z^*)$  for the areas of  $W^*$  and  $Z^*$ , then we have the following result (a generalization of the lemma of Schwarz):



## THEOREM 2.\*

$$|f'(0)| < \sqrt{\frac{V(Z^*)}{\pi}} \cdot \sqrt{\frac{V(W^*)}{\pi}}, \quad (3.1)$$

unless  $f(z) = a_1 z$ .

We write  $D^*$  for the "star" of a domain  $D$  with respect to the origin, †  $\bar{D}$  for the closure of  $D$  (i.e.,  $\bar{D} = D + \text{boundary of } D$ ), and write  $D^{-1}$  for the set of points  $R^{-1}e^{i\phi}$ , where  $Re^{i\phi} \in D$ . We denote the  $z$ -plane, including the point  $z = \infty$ , by  $P_z$ , and define

$$Z^{**} = P_z - \overline{\{(P_z - \bar{Z}^*)^{-1}\}^*}^{-1}.$$

Then  $Z^* \subset Z^{**}$ . Bermant‡ has proved that

$$|f'(0)| \leq \sqrt{\frac{V(Z^{**})}{\pi}} \cdot \sqrt{\frac{V(W^*)}{\pi}}, \quad (3.2)$$

with equality only for  $f = a_1 z$ .

To prove Theorem 2, we need only apply Theorem 1 at the appropriate point in Bermant's proof of (3.2) (Bermant, *loc. cit.*). However, for the sake of completeness, I include a proof of Theorem 2 using a method§ different from that of Bermant.

We may plainly suppose that  $|f'(0)| = 1$ . We write  $\zeta = \xi + i\eta$ . Suppose that a function  $g(\zeta)$  maps a Riemann domain  $G_1$  on a (Riemann) domain  $G_2$ . Let  $q(\eta)$  be a set of points of  $G_1$ , defined for each  $\eta$  of a set  $H$ , which lie over the line  $\eta = \text{constant}$ , and write

$$E_1 = \sum q(\eta), \quad V_1 = mE_1, \quad V_2 = \int_{E_1} \int |g'(\zeta)|^2 d\xi d\eta$$

$$l(\eta) = g\{q(\eta)\}, \quad L(\eta) = ml(\eta) = \text{"length" of } l(\eta).$$

Then we have:

\* Theorem 2 is stated without proof in my paper (reference 5).

† But  $Z^*$  is *not* a star; this will lead to no confusion.

‡ Bermant (see reference 1 at end of paper). The first result of this kind is due to Golusin (see reference 2), who proved that  $|f'(0)| < \sqrt{\frac{V(W^*)}{\pi}}$ .

§ See Spencer (reference 5), where the method is applied to multiply-connected domains.

LEMMA. Suppose that  $L(\eta) \geq \lambda$  for almost all  $\eta \in H$ . Then

$$(\lambda \cdot mH)^2 \leq V_1 V_2$$

(and there is equality only if  $V_1 = 0$  or  $g = a_0 + a_1 \zeta$ ).

I omit the proof (which is two lines in length); it has been given elsewhere\* in any case.

We suppose that  $r$  is small enough that the closed circle  $|w| \leq r$  is contained in  $W^*$ . We remove this circle from  $W^*$ , and cut the remaining domain along the real axis from  $w = r$  to the nearest boundary point to form a simply connected domain  $W^*_{,r}$ . We write  $Z^*_{,r} = f^{-1}(W^*_{,r})$ , transform  $W^*_{,r}, Z^*_{,r}$  by  $\zeta = \lg w, \sigma = \lg z$  into two domains  $G_1(r), G_2(r)$ , respectively, and apply the lemma. We take for  $H$  the interval  $(0, 2\pi)$ , for  $q(\eta)$  those points of  $G_1(r)$  which lie on the line  $\eta = \text{constant}$ . Then  $q(\eta)$  is (for every  $\eta$  of  $H$ ) a line segment connecting the logarithmic transform of some boundary point of  $W$  to the straight line  $\zeta = \lg r$ . Therefore the transform  $l(\eta)$  of  $q(\eta)$  connects the line  $\text{Re}(\sigma) = 0$  to a curve lying inside the rectangle

$$\lg r - \epsilon(r) \leq \text{Re}(\sigma) \leq \lg r + \epsilon(r), 0 \leq \text{Im}(\sigma) \leq 2\pi,$$

where  $0 < \epsilon(K \cdot r) < 0(r)$ , and so  $L(\eta) \geq \lg \frac{1}{r} - \epsilon(r) = \lambda$ . By the lemma we have, therefore,

$$2\pi \left( \lg \frac{1}{r} - \epsilon(r) \right) \leq \sqrt{V_1(r) \cdot V_2(r)} \leq \frac{V_1(r) + V_2(r)}{2}, \quad (3.3)$$

where  $V_1(r)$  and  $V_2(r)$  are the areas of  $G_1(r)$  and  $G_2(r)$ , respectively. Let  $p_1^*(R), p_2^*(R)$  be the functions defined by (1.1) in terms of  $W^*, Z^*$ , respectively. Then

$$V_1(r) = \int_r^\infty p_1^*(R) d(2\pi \lg R) = 2\pi \lg \frac{1}{r} + \pi \int_r^\infty \lg R^2 d[-p_1^*(R)], \quad (3.4)$$

and

$$V_2(r) = \int_r^\infty p_2^*(R) d(2\pi \lg R) + 0(r) = 2\pi \lg \frac{1}{r} + \pi \int_r^\infty \lg R^2 d[-p_2^*(R)] + 0(r). \quad (3.5)$$

\* Spencer (reference 5).

Substituting from (3.4) and (3.5) into (3.3), and letting  $r \rightarrow 0$ , we obtain that

$$0 \leq \int_r^\infty \lg R^2 d[-p_1^*(R)] + \int_r^\infty \lg R^2 d[-p_2^*(R)]. \quad (3.6)$$

Since both  $W^*$  and  $Z^*$  satisfy the hypothesis  $(H)^\dagger$  with  $n_0 = 1$ , we may take the exponent of both sides of (3.6) and apply Theorem 1, obtaining (3.1). There is equality (by Theorem 1) only if  $W^*$  is a circle—i.e., only if  $f = a_1 z$ . This completes the proof of Theorem 2.

Inequality (3.6) (in different notation) is due to Bermant (loc. cit.). We note that  $d[-p_1^*(R)] \geq 0$ , but the corresponding statement for  $p_2^*(R)$  is in general false. It is for this reason that the ordinary version of the theorem of the arithmetic and geometric means (in which the weighting is assumed positive) cannot be applied.

<sup>1</sup> Bermant, A., "Remarque sur le lemme de Schwarz," *C. R. Acad. Sci. Paris*, **207**, 31–33 (1938).

<sup>2</sup> Golusin, G. M., "Einige Überdeckungssätze für die im Kreise regulären Funktionen," *C. R. Acad. Sci. U. R. S. S.*, **2**, 617–619 (1937).

<sup>3</sup> Grunsky, H., "Neue Abschätzungen zur konformen Abbildung ein- und mehrfach zusammenhängender Bereiche," *Schr. math. Sem. Univ. Berlin*, **1**, 95–140 (1932).

<sup>4</sup> Spencer, D. C., "On finitely mean valent functions," *Proc. London Math. Soc.* (to appear shortly).

<sup>5</sup> Spencer, D. C., "On a Theorem of Rengels," *Journal of Math. and Physics, Mass. Inst. of Technology* (to appear shortly).

<sup>†</sup> In fact, both satisfy the stronger hypothesis that  $n(w) \leq 1$ ; we require, therefore, only Grunsky's theorem.

## MINIMAL CROSS-CUT SUBGROUPS RELATIVE TO THE PRODUCT OF THEIR ORDERS

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If  $H_1$  is a subgroup of a given group  $G$  then according to E. Galois (1811–1832) all the operators of  $G$  can be represented in the form of right co-sets or in the form of left co-sets with respect to  $H_1$ . This is equivalent to the earlier arrangement by P. Abbati (1768–1842) of all the operators of  $G$  in the form of a rectangle either with respect to right or left multiplication in which the operators of  $H_1$  constitute the first row and always appear on the same side in the products. Let  $H_2$  be any other subgroup of  $G$  which is not

contained in  $H_1$ . The operators of  $H_2$  will then be equally distributed in the given rectangular arrangement of the operators of  $G$  among the rows in which at least one operator of  $H_2$  appears. That is, each of these rows contains either no operator of  $H_2$  or it contains just as many operators of  $H_2$  as appear in the first row.

When  $H_2$  coincides with  $G$  then the operators of  $H_2$  appear in all of the rows of  $G$  and this may also be the case when  $H_2$  is a proper subgroup of  $G$ , that is, when  $H_2$  is neither the identity nor the entire group. In what follows the term subgroup will always be used for a proper subgroup unless the contrary is explicitly stated. A necessary and sufficient condition that the operators of  $H_2$  appear in all of the rows of  $G$  in the given arrangement is that the product of the orders of  $H_1$  and  $H_2$  divided by the order of their cross-cut is equal to the order of  $G$ . When this condition is satisfied  $H_1$  and  $H_2$  may be said to have a minimal cross-cut with respect to the product of their orders or to be minimal cross-cut subgroups relative to the product of their orders. \*It is obvious that the product of the orders of two subgroups divided by the order of their cross-cut cannot exceed the order of the group. It is a multiple of the order of each of these subgroups and hence composite.

When  $G$  is the non-cyclic group of order  $p^2$ ,  $p$  being a prime number, or when it is the cyclic group whose order is the product of two distinct prime numbers then every pair of subgroups of  $G$  is a pair of minimal cross-cut subgroups relative to the product of their orders. It is not difficult to prove that no other group has this property. It is obvious that the order of such a group could not be of the form  $p^m$ ,  $m > 2$ , since it could not be cyclic because one of the two subgroups could not appear in the other as  $H_2$  was supposed to be not contained in  $H_1$ . It could therefore not be the group of order  $2^m$  which contains only one subgroup of order 2. Since every other non-cyclic group of order  $p^m$  contains the non-cyclic group of order  $p^2$  it has been proved that the order of  $G$  could not be  $p^m$ ,  $m > 2$ . As the order of  $G$  could not be divisible by three distinct prime numbers or by the square of a prime number when it is divisible by just two distinct prime numbers it has been proved that *the non-cyclic group whose order is the square of a prime number and the cyclic group whose order is the product of two distinct prime numbers are the only groups which have the property that every pair of their subgroups is a pair of minimal cross-cut subgroups with respect to the product of the orders of these subgroups.*

When operators of  $H_2$  appear in all of the rows of  $G$  with respect to  $H_1$  the operators of  $H_1$  appear in all of the rows of  $G$  with respect to  $H_2$  so that the property of minimal cross-cut subgroups is a reciprocal property. If one of a pair of minimal cross-cut subgroups relative to the product of their orders is of prime order then the other is of the same prime index. It could not be of a larger index since its order is a divisor of the order of the group,

and it could not be of a smaller index since the product of the orders of the two subgroups divided by the order of their cross-cut is equal to the order of the group. In particular, if one of two minimal cross-cut subgroups relative to the product of their orders is of order 2 the other is an invariant subgroup of index 2, and if one of two minimal cross-cut subgroups with respect to the product of their orders is of index 2 then every other subgroup of the group forms with this subgroup a pair of minimal cross-cut subgroups. In particular, the minimal cross-cut subgroups with respect to a given subgroup may have different orders and some other subgroups may have a smaller cross-cut.

Suppose that  $H_1$  and  $H_2$  are two conjugate subgroups of  $G$ . It is then easy to prove that  $H_1$  and  $H_2$  cannot have a minimal cross-cut relative to the product of their orders. If they had such a cross-cut the operators of  $H_2$  would be equally distributed among all the rows of  $G$  with respect to the rectangle in which  $H_1$  is the first row and the operators of  $H_1$  appear on the left in the products which constitute the other rows. All the operators of each of these rows would transform  $H_1$  into the same one of its conjugates. These could not include  $H_2$  since each row may be assumed to begin with an operator of  $H_2$  and hence cannot transform  $H_1$  into  $H_2$  since it transforms  $H_2$  into itself. It therefore results that the cross-cut of any two conjugate subgroups of  $G$  is larger with respect to the product of their orders than the index of one of these subgroups under the entire group. That is, the quotient of this product of these orders and the order of this cross-cut is less than the index of one of these conjugate subgroups under the entire group.

If  $G$  is a multiply transitive permutation group of degree  $n$  and  $H_1, H_2$  are two subgroups composed of all its permutations which omit a given letter in each case then  $H_1$  and  $H_2$  are known to be conjugate and if the permutations of  $H_1$  constitute the first row when  $G$  is written in the ordinary rectangular form then the permutations of  $H_2$  will appear in  $n - 1$  of the rows, since  $H_1$  and  $H_2$  are transitive. Moreover, if  $G$  is not multiply transitive then the permutations of  $H_2$  cannot appear in as many as  $n - 1$  of these rows because the number of rows in which these permutations appear is equal to the degree of a transitive constituent of  $H_1$  and this number cannot exceed  $n - 3$ . The property of multiple transitivity as well as the degree of the various transitive constituents of  $H_2$  can therefore be directly observed from the number of rows in which the permutations of  $H_2$  appear with respect to  $H_1$ .

A cyclic prime power group does not contain a pair of minimal cross-cut subgroups relative to the product of their orders since one of every two subgroups of such a group is contained in the other. Every other abelian group contains at least one pair of such subgroups since it is the direct product of two cyclic groups which have only the identity in common. Moreover, every non-cyclic prime power group contains a pair of minimal



cross-cut subgroups relative to the product of their orders since such a group of order  $p^m$  contains two distinct subgroups of index  $p$  which can be used for  $H_1$  and  $H_2$ , respectively. Since a solvable group necessarily contains an invariant subgroup of prime index this subgroup and an operator which is a power of this prime number but is not contained in this subgroup can be used as a pair of minimal cross subgroup relative to the product of their orders whenever this solvable group is not a cyclic group of prime power order.

The symmetric group of degree  $n > 3$  contains a regular group which is one of a pair of minimal cross-cut subgroups with respect to the product of their orders if the other is the symmetric group of degree  $n - 1$ . This is also true of the alternating group of degree  $n$  whenever  $n$  is odd. When  $n$  is even and equal to  $2m$  we can construct a group of order  $2^m m!$  and of degree  $n$  which contains a subgroup of index 2 composed of its positive permutations which with the alternating group of degree  $n - 1$  constitutes a pair of minimal cross-cut subgroups with respect to the product of their orders. These two subgroups have in common a group of order  $2^{m-1} m!$  divided by  $n$  and hence the product of their orders is  $n!$  over 2. Therefore every symmetric group and every alternating group contains a pair of minimal cross-cut subgroups relative to the product of the orders of these subgroups.

One of the most important properties of two minimal cross-cut subgroups relative to the product of their orders is that they cannot be conjugate under the group. If one of these two subgroups and their cross-cut are invariant under the group the other subgroup is isomorphic with the quotient group with respect to this invariant subgroup. If both of these minimal cross-cut subgroups are invariant under the group the commutators which have one element from each of these subgroups are in their cross-cut and this cross-cut is also invariant under the group. If this cross-cut is the identity then every operator of one of these subgroups is commutative with every operator of the other and hence the group is the direct product of these two minimal cross-cut subgroups relative to the product of their orders. For instance, a group whose order is divisible by two and only two distinct prime numbers which has invariant Sylow subgroup of different orders is the direct product of two such subgroups.

The difference between the product of the orders of two subgroups and the order of the group is divisible by the order of each of these subgroups. When this difference is zero and the orders of these subgroups are relatively prime they are minimal cross-cut subgroups relative to the product of their orders. The product of the orders of two subgroups of a group cannot exceed the square of the order of the group divided by 4 and when it is equal to this number the group contains an invariant subgroup of index 4 corresponding to the non-cyclic group of order 4 as a quotient group. In par-



ticular, the group contains then at least three subgroups of index 2 and the two given subgroups are minimal cross-cut subgroups relative to the product of their orders. *The square of the order of a group divided by the product of the orders of two subgroups is a composite number and when this number is divisible by only two prime numbers (equal or distinct) these subgroups are necessarily minimal cross-cut subgroups relative to the product of their orders.*



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*THE INFLUENCE OF NERVE FIBERS UPON TASTE BUDS  
DURING EMBRYONIC DEVELOPMENT\**

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It has long since been demonstrated in a variety of animal forms that subsequent to experimentally induced degeneration of gustatory nerve fibers the taste buds associated with them disappear. It has also been shown that when the nerve fibers regenerate, new buds make their appearance. Taste buds, therefore, are intimately dependent for their existence upon their nervous supply; and by way of explaining the possible physiological nature of this dependence, a neurohumoral mediator has been hypothesized (Torrey, '34a, '36).

On the basis of accounts given by Hermann ('84), Gräberg ('98) and others, it has been inferred that nerves probably play a similar rôle in the ontogeny of gustatory organs. Hermann, for instance, maintained that nerve fibers came into contact with the epithelium of the developing papillae on the tongue of the foetal rabbit at the same time the anlagen of the taste buds were appearing, and thus nerves were presumed to have stimulated or induced the formation of the sense organs. Szymonowicz ('95, '96) made a similar suggestion for the Corpuscles of Grandry and Herbst, and more recently Whiteside ('26), speaking of the regeneration of taste buds in the rat, has said that inasmuch "as the regenerative process resembles the embryonic, the causative factor would probably be the same in both cases."

Other workers, by contrast, have not accepted this inference. Herbst ('01) and Harrison ('04), for example, have said that the coincidental appearance of nerve fiber and sense organ is not proof of the relation of cause and effect. Harrison has gone further and like Roux differentiated between a "function of development" and a "function of conservation;" that is, the power to differentiate is inherent within the anlage itself, whereas the stimulus that subsequently maintains the organ is supplied by the nerve.

This conception is furthered by Patzelt ('24) who maintains that not only is the ability to differentiate into taste buds inherent within the epithelium, but that the nerve fibers actually grow in secondarily. And finally, Stone ('33, '40) concludes from his studies of grafted tongue primordia in *Amblystoma* that in this form, at least, nerves do not play a causative rôle in the formation of taste buds.

Obviously the intimate dependence of taste buds upon their nerve supply exhibited during degeneration and regeneration in the adult individual has not been demonstrated to exist for certain during ontogeny. An attempt has been made, therefore, to solve this long-time problem. The investigation has followed two courses. The first consisted of working out the normal embryogeny of the sense organs and their nerve fibers. This was necessarily prerequisite to the second: an experimental analysis of the morphogenetic association prevailing between the two. All experiments and observations have been performed on the rat and attention directed solely towards the vallate papilla of which there is a single one only in the rat.

The development of the vallate papilla (as of all the other papillae) precedes that of the taste buds by a considerable time. The single papilla originates in a manner so similar to that already described for those of the rabbit (Hermann, '84) and man (Gräberg, '98), that additional description is hardly warranted. Suffice it to say for the rat that the papilla begins to take form towards the end of the fifteenth day of gestation and is completely established by the third day postpartum. The first taste buds, however, do not appear until about the ninth day after birth. This cannot be said to represent an absolute date, for some individual variation occurs. In one or two instances, for example, an occasional bud was to be seen early on the eighth day, whereas on other occasions buds could not be detected until the tenth. Generally speaking, however, the time of initial appearance of the buds is the ninth day. Few in number at this time, they gradually multiply, reaching a maximum in about twelve weeks, from which time they undergo a slight reduction in numbers until an adult constant of roughly 75 to 100 buds, associated exclusively with ventral levels of the papilla and bounding trench, is attained.

Special attention was directed towards the time of arrival of the ingrowing nerve fibers at their epithelial terminals. For this purpose whole embryos and pieces of tongues ranging from a prenatal age of fifteen days to twelve days postpartum were fixed in formol-acetic-alcohol and stained with activated protargol (Bodian, '37).

Other workers have shown that the single anlage of the glossopharyngeal ganglia is established towards the beginning of the twelfth day of gestation and promptly divides into a dorsal group of cells, the future superior ganglion, and a ventral group, the future petrosal ganglion. Processes are soon

given off from the neuroblasts and by the end of the thirteenth day a definitive glossopharyngeal nerve has connected centrally with the brain and is pushing peripherally towards its final terminations.

My own observations reveal that as soon thereafter as the beginning of the sixteenth day a fairly conspicuous bundle of fibers is present in the basal portions of the developing tongue lying not far beneath the dorsal epithelium. These fibers continue peripherally during the succeeding two days, reaching and even extending beyond the level of the already developing vallate papilla. Sections of a twenty-day tongue reveal nerve fibers which have entered and traversed the subepithelial tissues of the papilla to terminate in the form of a subepithelial plexus. From this time until the first taste buds appear some ten days later the number of fibers within the papilla, and thus at the same time the extent of the subepithelial plexus, are increased by the arrival of new bundles of fibers from various directions beyond the papilla.

A few of the preparations of postpartum stages previous to the ninth day show occasional fibers which have penetrated the papillary epithelium. Such intraepithelial fibers are so difficult to demonstrate histologically that an accurate count of their absolute number has not been attempted. Sufficient of them are stained, however, to demonstrate unquestionably that such *fibers are present several days in advance of taste bud differentiation.*

If one is committed to the proposition that nerves do incite or determine the formation of taste buds, then the above facts lend themselves in support. The precocious arrival of the fibrils at the site of the future end organs may, however, be entirely without significance other than that a nervous supply is ready and waiting for the buds when they do appear, for it is yet to be demonstrated that the fibers literally exert a "stimulus of development" which brings the end organs into existence. A critical test would consist in setting up conditions whereby the buds would be permitted, if they are capable of doing so, to develop free from the influence of nervous elements. This has been attempted in two entirely different fashions: first, by causing a previously destroyed papilla in the adult to regenerate in the absence of functional nerve fibers; second, by transplantation of papillae of pre-taste bud ages.

Whiteside ('26) has shown that following complete extirpation, the vallate papilla of the rat begins to regenerate within three weeks and in six months is completely restored. New taste buds likewise develop in the epithelium of the regenerating papilla, the first buds usually appearing about the same time the papilla proper begins to take form, i.e., at three weeks, and continuing to be formed for as long as twenty-two weeks.

In the light of the known relations of gustatory organs to degenerating and regenerating nerves, buds are formed out of the newly established epithelium of a regenerating papilla presumably under the influence of the

gustatory fibers likewise resupplying the papilla. Assuming that the regeneration process is closely akin to the original ontogeny, a test of the taste bud forming capabilities of a regenerating papilla *devoid* of functional nerves might be expected to give a partial answer to the question of the ontogenetic relation of the sense organ and nervous supply. The conditions for such a test have been set up by destroying the vallate papilla and cutting the glossopharyngeal nerves which supply it.

Four to seven months old male and female albinos were used for most of these experiments, supplemented by a few individuals from a chocolate hooded stock employed for the transplantation experiments to be described later. Individuals were anesthetized with sodium amytal and the vallate papilla cauterized. Because most of the buds have a bilateral innervation (Whiteside, '27) it was necessary to cut both glossopharyngeal nerves. Each was located where it lay dorsal to the anterior belly of the digastricus muscle and severed proximal to the junction of its pharyngeal and lingual branches.

Fifteen animals were operated upon in this fashion. For controls fifteen animals with the papillae alone destroyed were used. Three experimental and three control animals were sacrificed two weeks after operation and the tongues prepared for study. Additional groups of three each were sacrificed so as to comprise a series with the groups separated by four-day intervals. There was thus available material of fourteen, eighteen, twenty-two, twenty-six and thirty days post-operative ages.

At the fourteen-day stage in both the experimental and control animals, the regeneration process has gone little beyond the wound healing stage. The papilla has not yet begun to reform and there are no taste buds present. Four days later there are evidences of the epithelial proliferation preceding the formation of a definite papilla. The papilla gradually begins to take form during the succeeding stages in both the experimental and control animals. The details of its regeneration conform to those already given by Whiteside. The one additional contribution made by the present investigation is that regeneration of the papilla goes on independent of the nervous system; the known dependence of regenerating taste buds on nerve fibers does not prevail for the papilla. It is interesting to note in this connection that the barbels of the catfish, by contrast, will not regenerate in the absence of a normal nerve (Olmsted, '20).

As far as the buds themselves are concerned, the first newly formed ones were observed in the control animals of twenty-six days. There were three buds in one of these, and five in the other two. The three controls of the thirty-day stage showed twelve buds in one, thirteen in the second and fifteen in the third. These observations on the number and time of first appearance of the buds conform fairly closely to those recorded by Whiteside.



In the experimental animals, by contrast, buds were observed in only one instance, that of one of the three thirty-day individuals, and in this case two well-defined buds were seen. There was reason to believe, however, that regenerating nerve fibers from the central trunk may have reached the papilla and induced the buds to form out of the regenerating papillary epithelium. To test this likelihood the papillae of four animals were cauterized at one time and the nerves severed ten days later. This was considered an interval sufficient to prevent the normal nerves from exerting any influence upon the reforming papilla while at the same time shortening the period available for the return of the new fibers. Two of the animals were sacrificed thirty days after cautery and two at thirty-five days. There were no taste buds present in any of the four.

The conclusion to be derived from these experiments, then, is that in the adult individual *taste organs regenerate only under the influence of nerve fibers*. To carry this conclusion over to the embryonic origin of the buds, however, requires the assumption that the regeneration of an organ is strictly comparable to its ontogeny, an assumption not wholly justified by the comparative data of regeneration phenomena in general. It remains to be demonstrated that this "function of conservation" on the part of nerves exists as a "function of development." The following experiments comprise an attempt at such a demonstration.

A crucial test of the ability of the developing tongue to give rise to taste buds in the absence of nervous stimulation involves setting up conditions whereby embryonic differentiation of the papilla would continue in an environment totally devoid of nervous elements. It was believed these conditions would be found in some variety of graft.

Preliminary experiments showed that subcutaneous, omental and ear grafts were unsuitable for several reasons. Not only did the transplanted tissues degenerate rather rapidly, but were subject to distorting pressures and were commonly invaded by considerable amounts of fat and connective tissue. There was the ever-present possibility, too, that such grafts, even if they survived and continued differentiation, would be invaded by nerve fibers from the surrounding host tissues. This eventuality alone would defeat the purpose of the experiment. It was necessary, then, to employ an environment of a more "neutral" character. To this end resort was had to the anterior chamber of the eye, a site offering many of the advantages of explanation methods and few of the technical difficulties.

The material, host and donor, for such grafts was derived from a strain of chocolate hooded rats inbred for 25, 26 and 27 generations. The original 25th generation stock was obtained through the courtesy of Dr. R. T. Hill of the Indiana University Medical School. Later in the project this material was supplemented by Sprague-Dawley albino hosts and donors which proved to be equally satisfactory. The transplants were obtained from

donors ranging from a foetal age of sixteen days to eight days postpartum (the interval during which the vallate papilla and its nervous supply develop, but terminating just short of the time of the initial appearance of taste buds). The age of the host animals ranged from 120 to 212 days.

The accompanying table summarizes the history of the grafts.

TABLE 1

EXPT. NO.	NO. OF RECOVERED GRAFTS	DONOR	AGE OF GRAFT (DAYS AFTER OPBR.)	THEORETICAL POST-PARTUM AGE	AGE BEYOND NORMAL TASTE BUD THRESHOLD	TASTE BUDS
Te-1	5	1 day p. p.	10 days	11 days	2 days	None
Te-2	6	4 day p. p.	15 "	19 "	10 "	None
Te-3	4	18 day foetus	20 "	15 "	6 "	None
Te-4	5	18 day foetus	14 "	11 "	2 "	None
Te-5	8	6 day p. p.	6 "	12 "	3 "	None
Te-6	10	8 day p. p.	3 "	11 "	2 "	Present in all
Te-7	8	3 day p. p.	8 "	11 "	2 "	None
Te-8	4	20 day foetus	11 "	10 "	1 "	None
Te-9	7	7 day p. p.	5 "	12 "	3 "	Present in 3 cases

It will be observed that in all the groups of experiments the grafts were permitted to grow for a period of time in excess of that normally required for the initial appearance of taste buds. Group Te-4, for example, comprises transplants of an original foetal age of eighteen days cultivated for two weeks. Three days out of those two weeks can be thought of as belonging to the gestation period; thus the recovered graft is at a theoretical age of eleven days postpartum. It will be recalled that this age is two days beyond the average ninth-day threshold at which taste buds normally appear. Likewise the other groups of transplants, with one exception, were allowed to attain post-threshold ages of two or more days (cf. table). The exception is group Te-8 which approaches within one day of the average ninth-day threshold, but it is still within the range of observed variation in normal time of taste bud appearance.

As far as the time element alone is concerned, therefore, taste buds should have developed in all the grafts if they were capable of spontaneously doing so. On the contrary, buds appeared in only two groups of transplants; those grafted at eight (Te-6) and seven days (Te-9) of age. In the former, from two to five buds were found in all ten of the recovered grafts; buds were found in only three out of seven cases of the latter group.

This immediately suggests that determination of the taste buds, whatever the mechanism thereof, occurs between the seventh and eighth days after birth. When it is recalled that nerve fibers normally arrive at the site of future taste buds not later than the twentieth prenatal day, it appears possible that they have been involved in the determination process. However, their "function of development," if they perform such, must not

come into play before the seventh day, otherwise taste buds should have appeared in the grafts of earlier ages.

It may still be argued, of course, that the determination mechanism is inherent within the papillary epithelium or involves some other unidentified factor, and later trophic associations with the nerve fibers are secondarily assumed. But if this were so, it is difficult to understand why gustatory organs do not appear in a graft of tissue of an early age which has been successfully cultivated beyond the bud threshold and in which the papillary epithelium has otherwise undergone an apparently normal developmental history. It may be stated in connection with this last point that a definite structural regression occurred only in the longer cultivated grafts of groups Te-2 and Te-3. The papillary epithelium in the other taste bud-free grafts appeared to have otherwise made normal developmental progress.

To recapitulate: Whereas nerve fibers arrive within a developing val-late papilla not later than the twentieth prenatal day, taste buds do not appear until some ten days later. These facts maybe interpreted as favoring the idea that nerves exert a stimulus or function of development; at least they are not contradictory, although they might be interpreted in other ways. More significant is the observation that taste buds will not reappear in a regenerating papilla unless their nervous supply is intact. But this can be considered a true function of development on the part of nerves only if it is first assumed that the regeneration of an organ is homologous to its ontogeny. The final set of experiments, therefore, lends the greatest support to the concept, for it is observed that in the nerve-free environment of the anterior eye chamber taste buds fail to appear except in those papillae transplanted just previous to the time the buds normally appear. It is thus very probably true that nerve fibers do perform a function of development upon developing gustatory organs. It is recognized, however, that *the* critical experiment remains to be performed: the tongue itself of the developing animal must be left intact and undisturbed while its nerve supply in some fashion is prevented from reaching it. At the present time the technical problems barring this experiment have not been solved.

\*Contribution No. 286 from the Zoölogical Laboratories, Indiana University.

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## TOUCH-AND-GO PAIRING IN CHROMOSOMES

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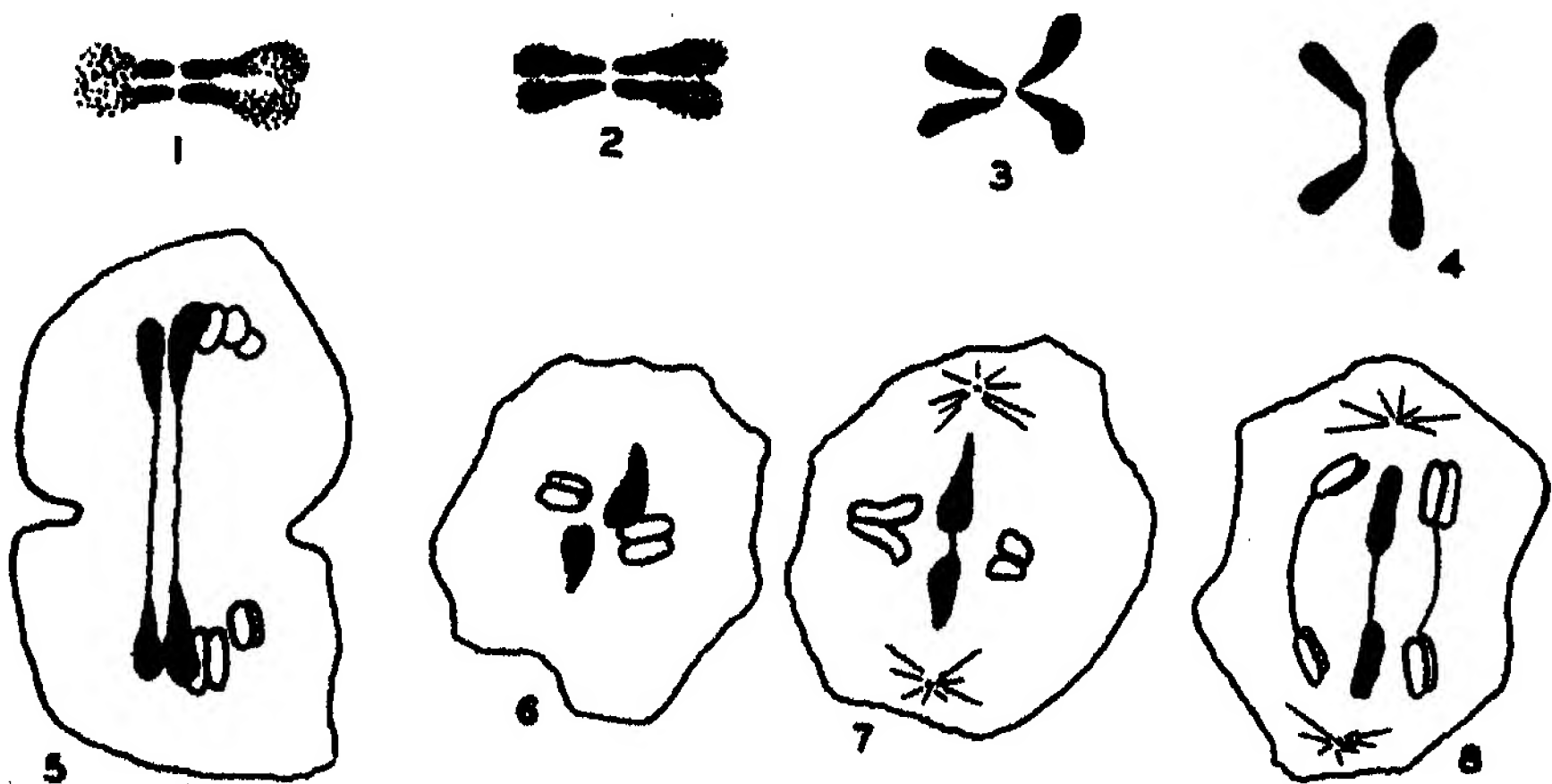
An orientation of chromosomes that appears to be independent of meiotic synapsis in the ordinary sense is shown in a relatively large number of species. Usually this takes the form of a vis-à-vis position on the spindle and when such chromosomes are separated by a considerable distance is called "distance conjugation" (Lorbeer, '34).<sup>1</sup> In other cases the involved chromosomes may actually come together for a period which in some instances is so brief that the movement has received the name "touch-and-go" process (Wilson, '25).<sup>2</sup>

The mechanism involved is a very puzzling one. In a general way the explanations that have been offered fall under two headings: (a) that some kind of attraction between the chromosomes is involved (Wilson, '32)<sup>3</sup> or (b) that it is mitotic forces ("centromere-spindle relationship") that bring about this orientation and that no specific attraction is involved (Darlington, '39).<sup>4</sup>

This latter explanation fails to account for the fact that in some instances such chromosomes approach each other before the spindle has been fully formed. Thus the *m* chromosomes in some cells of *Alydus* come to-

gether already in diakinesis (Reuter, '30),<sup>5</sup> and the  $XY$  pair of several pentatomids undergoes its brief union in the interkinesis or even the telophase prior to its disjunction. These cases seem to bespeak the existence of some kind of attraction and the following case lends additional support to such an argument.

In the male of the hemipter *Rhytidolomia senilis*—as has been pointed out elsewhere (Schrader, '40)<sup>6</sup>—the meiotic tetrads are formed by the terminal union of homologues in diakinesis. This is true also of the sex chromo-



*Rhytidolomia senilis*

Figures 1 and 2. The sex chromosomes in diakinesis. They show the equational split and have come together terminally at their euchromatic ends. The heterochromatic ends are not fully condensed. The larger  $X$  is on the right.

Figures 3 to 5. Progressive stages in the division of the sex chromosomes in the first division. Poleward movement occurs with the large end of heterochromatic end foremost. The  $X$  is on the right.

Figure 6. Late telophase of interkinesis with  $X$  and  $Y$  beginning to show orientation toward each other.

Figure 7. Metaphase or early anaphase showing the large ends of  $X$  and  $Y$  oriented toward each other. Probably they are here already beginning to separate.

Figure 8. Late anaphase showing the loss of the club shape in the sex chromosomes.

somes; but it is to be noted that the  $X$  and  $Y$  chromosomes are each composed of a euchromatic and a heterochromatic section and that their diakinetic union always occurs at the euchromatic ends. This fact can be determined with little difficulty by tracing the chromosomes through the prophases. But it can also be demonstrated in the fully condensed chromosomes because both the  $X$  and the  $Y$  are clubshaped and the narrow end where the union takes place represents the euchromatic section (Figs. 1 to 3).



The first division is, superficially at least, equational for both sex chromosomes. Their movement to the poles always occurs with the large or heterochromatic end foremost. Already in the initial steps of the division all connection between the *X* and *Y* disappears (Figs. 4 and 5), but in telophase or interkinesis they become reassociated. However, in contrast to the union that takes place during diakinesis, they now come together at their large or heterochromatic ends. The approach usually begins already in late telophase when the metaphase grouping of the chromosomes has become temporarily lost (Fig. 6). The final union appears to be nothing more than a contact. In some cells a tiny gap can be seen between the two chromosomes and is then often bridged by an achromatic connection—but it is difficult to decide whether such cases do not represent the initial step in the reductional separation of the *X* and *Y* in the second division (Fig. 7). In the late second anaphase the clubbed shape of both sex chromosomes is obliterated (Fig. 8).

It is natural to conclude that this interkinetic maneuver of the *X* and *Y* is in some way correlated with the properties of heterochromatin. But whether it is or not does not touch the question here at issue. Also it may be granted that the interplay of mitotic forces is to some degree acting in any grouping of chromosomes on the spindle (distance conjugation makes this almost certain). But by the same token the *Rhytidolomia* case furnishes strong evidence that the touch-and-go movement involves not only an attraction between the *X* and *Y*, but an attraction of a very specific type.

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<sup>2</sup> Wilson, E. B., *The Cell*, pp. 1–1232 (1925).

<sup>3</sup> Wilson, E. B., *Jour. Morph.*, **53**, 443–468 (1932).

<sup>4</sup> Darlington, C. D., *Jour. Genet.*, **39**, 101–136 (1939).

<sup>5</sup> Reuter, E., *Act. Zool. Fenn.*, **9**, 1–484 (1930).

<sup>6</sup> Schrader, F., *Jour. Morph.*, **67**, 123–136 (1940).



## THE CORRECTION OF X-RAY DIFFRACTION INTENSITIES FOR LORENTZ AND POLARIZATION FACTORS

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1. *Introduction.*—In an earlier contribution,<sup>1</sup> a method was suggested for recording x-ray diffraction results as  $F^2$  values by direct photographic processes. This required the correction of the diffracted beams for Lorentz and polarization factors, and it was suggested that this could be accomplished with the aid of a rapidly rotating cam or shutter. A study of the theory of applying this correction has made it plain that, with the correct choice of variable, the form of the Lorentz factor is the same for all layers except for scale, and therefore a correction for it can always be made with the aid of a single instrumental cam regardless of the layer analyzed. On the other hand, the form of the polarization factor is different from level to level, and correction for it can best be made in another way. The results of this study can be applied not only in the recording of x-ray diffraction results as  $F^2$  values, but they are of immediate use in allowing for Lorentz and polarization effects in any method involving a rotating crystal completely immersed in an x-ray beam.

The Lorentz factor for single crystals was first given by Darwin<sup>2</sup> for the case of "equatorial" x-ray reflections. Cox and Shaw<sup>3</sup> subsequently showed how the Lorentz factor could be extended to non-equatorial reflections recorded by the normal-beam method. More recently, Tunell<sup>4</sup> has given a similar treatment in the extension of the Lorentz factor to non-equatorial reflections recorded by the equi-inclination method. A discussion of the Lorentz factor for the general-inclination case has only recently received attention by Bouman and de Jong.<sup>5</sup> They used a comparatively cumbersome method of deduction and unfortunately their results do not have a form which is very convenient to apply.

2. *General Characteristics of the Lorentz Factor.*—When a crystal is rotated in a beam of monochromatic x-radiation, the various planes of the crystal are caused to pass through positions in which they satisfy the condition for reflection. In general, the various planes do not occupy such positions for equal lengths of time. The total amount of x-radiation in each reflection is proportional to this time opportunity to reflect. In the several rotating crystal methods, the Lorentz factor is essentially this time factor.

The Lorentz factor is proportional to the time of reflection permitted to each reflection, or inversely proportional to the velocity with which the plane passes through the condition of reflection. Using  $L$  to represent the

Lorentz factor and  $V$  the velocity of passing through the reflecting condition for the reflection  $hkl$ ,

$$L_{hkl} \sim \frac{1}{V_{hkl}}. \quad (1)$$

The individual velocity of each reflection can be most conveniently investigated with the aid of the reciprocal lattice.

3. *Reciprocal Lattice Derivation.*—In figure 1 the important reciprocal lattice aspects of the problem are shown:  $A$  shows plan,  $C$  shows an elevation, while  $B$  shows an intermediate projection. The reciprocal lattice is rotating with an angular velocity,  $\Omega$ . Reciprocal lattice point,  $P$ , on the  $n$ th level, has just reached the  $n$  layer reflecting circle and is in the process of reflecting.  $P$  is moving in the direction  $PW$  and has an absolute velocity of  $\Omega\xi$ . The velocity with which it passes through the sphere, however, is the component of this velocity on the normal to the sphere at the point  $P$ , namely, its component on  $PS$ . Calling  $\eta$  the angle between  $PS$  and  $PW$ , the speed with which  $P$  passes through the condition of reflection is therefore given by

$$V = \Omega\xi \cos \eta. \quad (2)$$

To evaluate  $\cos \eta$ , pass a plane through the center of the sphere  $S$  and normal to the velocity direction,  $PW$ . It is evident that

$$\cos \eta = q. \quad (3)$$

Substituting this in (2) gives

$$V = \Omega\xi q. \quad (4)$$

Turning to figure 1- $A$ , it will be observed that the last two terms of (4) represent twice the area of triangle  $O_nPS_n$ . This same area can be represented alternatively by  $R_0p$ , where  $R_0$  is the radius of the zero-level reflecting circle. Substituting  $R_0p$  for  $\xi q$ , (4) becomes,

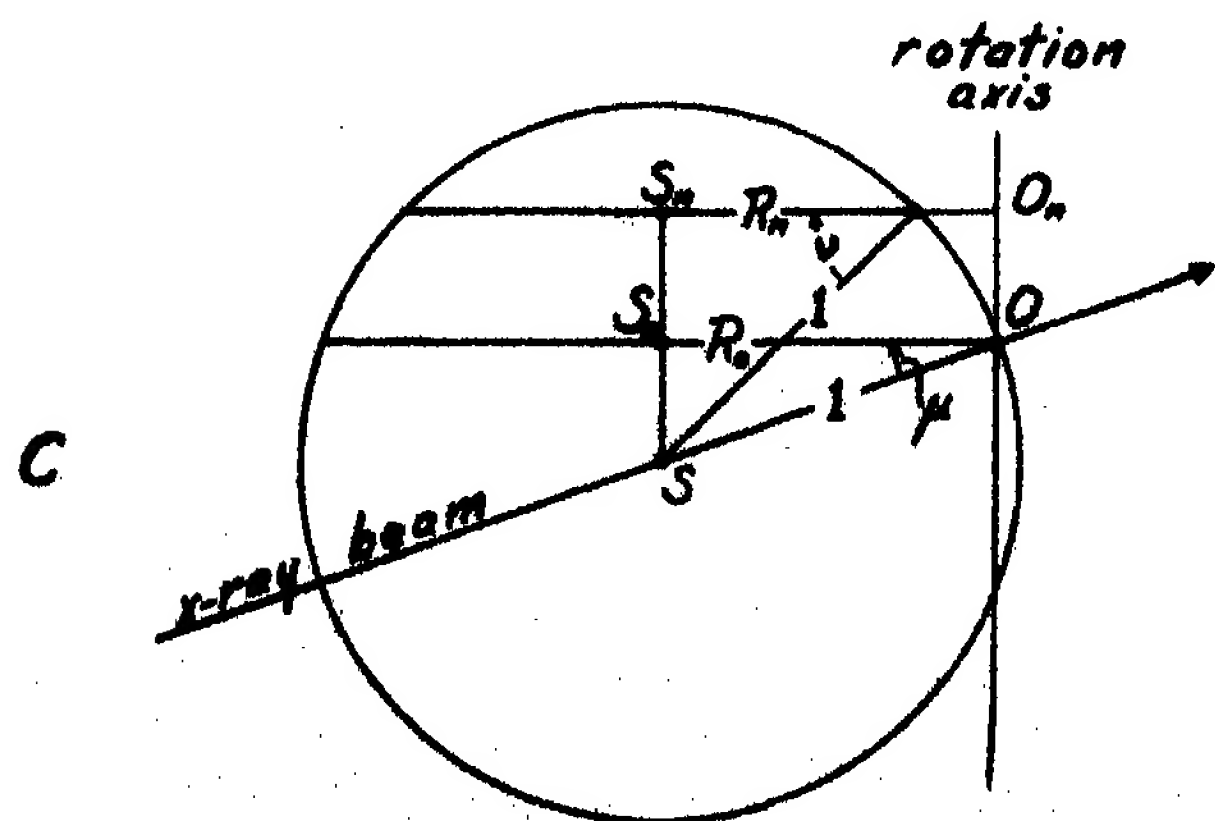
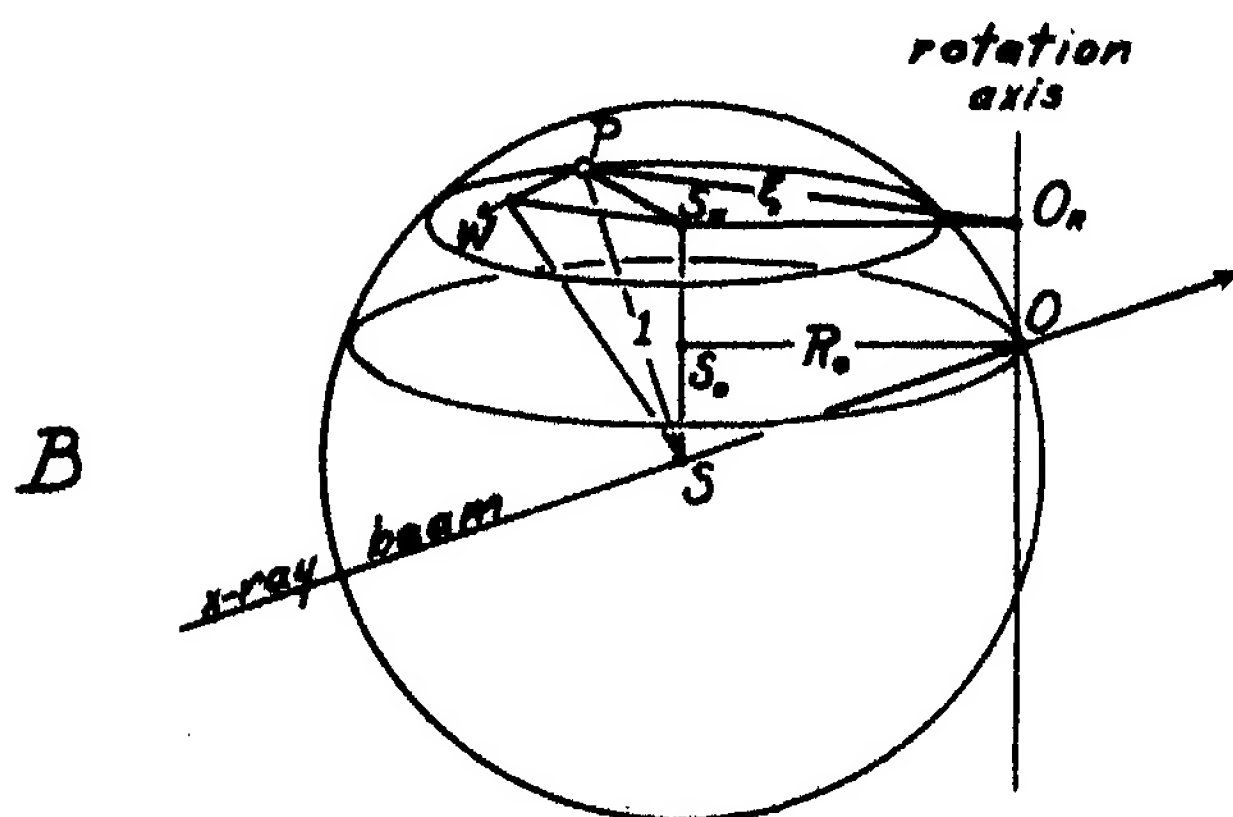
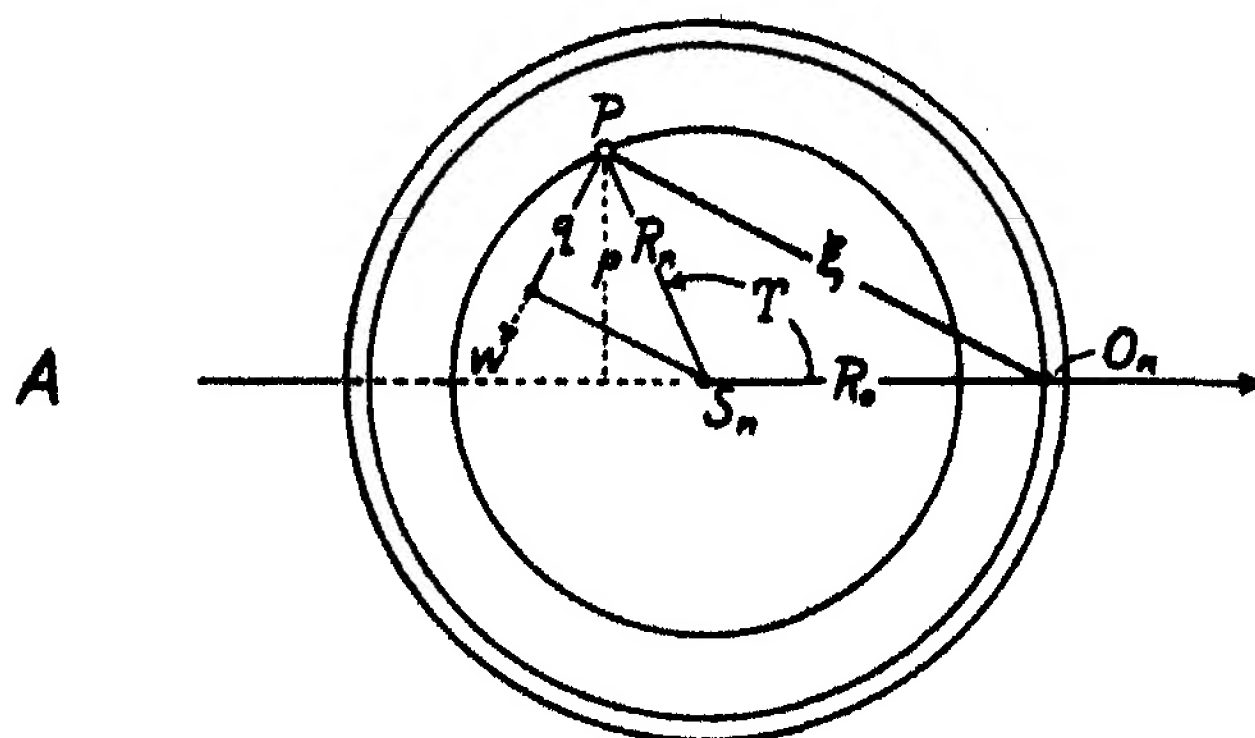
$$V = \Omega R_0 p. \quad (5)$$

This is in an inconvenient form. To transform it into something more useful, note that,

$$\sin \tau = \frac{p}{R_n}, \quad (6)$$

where  $R_n$  is the radius of the reflecting circle of the level containing  $P$ . Substituting in (5) the value of  $p$  indicated by (6) yields the required velocity in quite fundamental terms:

$$V = \Omega R_0 R_n \sin \tau. \quad (7)$$



**FIGURE 1**

Finally, the terms  $R_0$  and  $R_n$  may be expressed as functions of  $\mu$  and  $\nu$ , the angles which the direct x-ray beam and diffracted cone make with the levels of the reciprocal lattice. Figure 1-C shows that,

$$R_0 = \cos \mu, \quad (8)$$

and

$$R_n = \cos \nu. \quad (9)$$

Since  $\mu$  and  $\nu$  are values required for setting the instrument to record the layer, it is desirable to express  $V$  in terms of them. Substituting from (8) and (9) into (7) gives the required velocity in the desired form:

$$V = \Omega \cos \mu \cos \nu \sin \Upsilon. \quad (10)$$

The Lorentz factor for any condition of recording is now given by substituting the value of  $V$  from (10) into (1). The result may be simplified for practical experimental purposes by noting that the velocity of crystal rotation,  $\Omega$ , is common to all reflections; hence this term may be omitted from the proportion:

$$L \sim \frac{1}{\cos \mu \cos \nu \sin \Upsilon}. \quad (11)$$

This reduces to the following special cases for particular methods of recording reflections:

*Normal beam,  $\mu = 0$ :*

$$L \sim \frac{1}{\cos \nu \sin \Upsilon}. \quad (11A)$$

*Equi-inclination,  $\mu = -\nu$ :*

$$L \sim \frac{1}{\cos^2 \nu \sin \Upsilon}. \quad (11B)$$

*Flat-cone,  $\nu = 0$ :*

$$L \sim \frac{1}{\cos \mu \sin \Upsilon}. \quad (11C)$$

For equator reflections taken by the normal beam, equi-inclination or flat-cone methods,  $\mu = \nu = 0$ , and (11) can be reduced to the simple Darwinian form of the Lorentz factor:

$$L \sim \frac{1}{\sin \Upsilon} = \frac{1}{\sin 2\theta}. \quad (11D)$$

It is now most important to note that in (11) the pair of terms  $\cos \mu \cos \nu$  is constant for any level, recorded by any method. These terms may be called the scale of the Lorentz factor for the level. An important property

of (11) is that, except for scale, the form of the Lorentz factor is exactly the same for every layer and for every method of recording, provided that the comparison is made for the correct variable,  $\Upsilon$ .

4. *Application to Weissenberg Photographs.*—The vertical linear measure,  $x$ , of any Weissenberg photograph is directly proportional to the reflection azimuth,  $\Upsilon$ :

$$\Upsilon = \left[ \frac{360^\circ}{2\pi r_F} \right] x, \quad (12)$$

where the term in brackets is an instrumental constant dependent on the camera radius,  $r_F$ . In order to find the Lorentz factor for any spot on any Weissenberg film, therefore, the only measurement necessary is the distance,  $x$ , of the spot from the center line. Since the instrumental constant in (12) is exactly 2 for the standard Weissenberg camera radius, the angle,  $\Upsilon$ , can ordinarily be read directly with the aid of a millimeter scale. To find the Lorentz factor for the spot, it is merely necessary to take the cosecant of this angular reading and multiply it by the level scale in the form,

$\frac{1}{\cos \mu \cos \nu}$ , which is characteristic of the entire level and of the method of recording. Or if  $F^2$  is required, as for Fourier analysis, the correction is  $\sin \Upsilon$  multiplied by the scale  $\cos \mu \cos \nu$ . Either the Lorentz factor or the correction for it, disregarding the level scale, may be easily read for each spot on the film from a transparent chart (which is the same for all Weissenberg photographs) laid on or under the film.

5. *Elimination of Computation of Level-Scale Correction.*—Since the level-scale is constant for the entire level, it may be easily compensated for by deliberately arranging the time of exposure of each level so as to cancel it. The Lorentz factor varies from level to level by the scale,  $\frac{1}{\cos \mu \cos \nu}$ .

To compensate for this it is only necessary to expose each layer, regardless of how recorded, for a time proportional to  $\cos \mu \cos \nu$ . When this precaution is observed, all level photographs taken by any method whatever have the same Lorentz correction for equal values of  $\Upsilon$ .

6. *Elimination of Lorentz Corrections during Recording.*—In order to convert diffraction intensities into  $F^2$  values, it is necessary to apply a correction for both Lorentz and polarization factors. This is usually done by computation. The computation can be entirely avoided as far as the Lorentz factor is concerned by taking advantage of the semi-invariant form of the factor. Neglecting, for the moment, the matter of scale, we note that at  $\Upsilon = 90^\circ$  the Lorentz factor is unity and that it increases symmetrically above and below this value till it attains infinity at  $\Upsilon = 0^\circ$  and  $\Upsilon = 180^\circ$ . A correction for this Lorentz enhancement can be applied during recording by suppressing the enhanced reflections by a

factor which is the reciprocal of the enhancing (Lorentz) factor. This can be accomplished by reducing the time of recording of the more intense reflection with the aid of a rapidly rotating cam. The proper characteristics of the cam are that it has an opening proportional to  $\sin T$ . It is comparatively easy to arrange for such a cam in the method of de Jong and Bouman and also in the Sauter method. The writer has successfully applied it to de Jong and Bouman photographs and will provide further details of the method in another publication. On the other hand, it is difficult to arrange a rotating cam for the Weissenberg method, but it may be replaced here by a rapidly vibrating cylindrical shutter whose function is to differentially open and close the layer line screen slit. The design of such a shutter is complicated by the fact that the motion is reciprocating and hence is likely to be non-uniform.

The Lorentz correction may also be applied by absorbing reflections differentially as a function of  $T$ . The transmission of such an absorber should be proportional to  $\sin T$ .

Regardless of what method is used to reduce reflections by the factor  $\sin T$ , the Lorentz correction can be completely eliminated for any level and for any inclination scheme by simply arranging to have the exposure times for the levels proportional to  $\cos \mu \cos \nu$ , as discussed in section 5.

7. *Note on the Elimination of Polarization Factor Correction.*—The polarization factor can be eliminated by permitting transmission of reflections proportional to  $\frac{2}{1 + \cos^2 2\theta}$ . While this could be controlled with the aid of a cam, the function depends in an unfortunate way upon  $T$ ,  $\mu$  and  $\nu$ , so that a different cam would have to be used for each level and a new set for each new crystal. It should be noted, however, that the correction factor  $\frac{2}{1 + \cos^2 2\theta}$  has a range of only 1 to 2. It can therefore be easily applied by surrounding the crystal with a more or less spherical envelope of slightly absorbing material (such as a plastic) whose thickness variation has been designed to permit transmission of reflections by a factor  $\frac{2}{1 + \cos^2 2\theta}$ .

<sup>1</sup> Buerger, M. J., "The Photography of Interatomic Distance Vectors and of Crystal Patterns," *Proc. Nat. Acad. Sci.*, 25, 383-388 (1939).

<sup>2</sup> Darwin, C. G., "The Reflection of X-Rays from Imperfect Crystals," *Phil. Mag.*, 43, 808 (1922).

<sup>3</sup> Cox, E. G., and Shaw, W. F. B., "Correction Factors in the Photographic Measurement of X-Ray Intensities in Crystal Analysis," *Proc. Roy. Soc., (A)* 127, 71-88 (1930).

<sup>4</sup> Tunell, George, "The Rotation Factor for Equi-Inclination Weissenberg Photographs," *Amer. Mineral.*, 24, 448-451 (1939).

<sup>5</sup> Bouman, J., and de Jong, W. F., "Die Intensitäten der Punkte einer photographierten reziproken Netzebene," *Physica*, V, 9, 817-832 (1938).



*FUCUS CORDATUS TURNER*

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The *Fucus cordatus* of Turner (*Fuci*, 2, 118, 119, pl. 116 (1809)) has been a much misunderstood plant and its various misinterpretations have been responsible for other misconceptions. The species was described from specimens collected by Mr. Menzies at "Banks' Isles" on the northwest coast of North America on the second voyage of Vancouver. "Banks' Isles," now generally known as Banks Island, lies on the east side of Hecate Strait in British Columbia and at about 50° lat. N. and 130° long. W. The total distribution of this species, according to one or another author, extends from the northeastern coasts of Asia, around through the type locality, along the west Pacific Coast of North America to (or almost to) the southern boundary of the United States, the coasts of Chile to the Antarctic continent and up along the eastern coast of South America to (and including) the Falkland Islands (cf. particularly Gain, "Deux. Exp. Antarct. française," 1908-1910, "La Flore Algologique," 54-58, 1912) and even to South Africa. Such a range even among the Red Algae is almost unparalleled, but from the point of view of moderate polymorphism did not, until very recently, seem beyond plausibility.

*Fucus cordatus* was referred by Bory (*Dict. Class.*, 9, 16 (Feb., 1826)) to his proposed new genus *Iridea* as *Iridea cordata* and was the first of the binomials coined by him under this genus, although he mentioned the *Delesseria edulis* of Lamouroux first of all the species belonging to *Iridea*, but without coining a proper binomial for it. In Bory's time, fixing a type for a genus was not customary. Greville, however, in 1830 (*Alg. Britt.*, 136) definitely designates *Fucus edulis* Stackhouse as the type of the genus, as he had a perfect right to do. Later the name *Iridea* (or *Iridaea*) was more and more restricted to plants of the general type of *Fucus cordatus* and in 1847 J. G. Agardh (*Kongl. Vet. Akad.*, Handl., 82) in discussing the various types of South African plants still referred to *Iridea*, in his turn, formally designated *Fucus cordatus* Turner as the type of the genus, following the seeming usage of Bory in his latest account (*Voy. Cog., Bot.*, 2, 103-114 (Feb., 1828)) and of Kuetzing, who (*Phycol. gen.*, 395 (1843)) places *Fucus cordatus* first in the enumeration and in his illustration (loc. cit., pl. 77, II, not truly of *Fucus cordatus* Turner) of the genus, although he includes *I. edulis* (Stackh.) Grev. and others which J. G. Agardh (loc. cit.) finally excluded from his conception of *Iridaea*.

When the late Professor Nathaniel Lyon Gardner and myself, after somewhat over thirty years of collecting and study, issued our preliminary

reports (*Proc. Nat. Acad. Sci.*, 22, 469–473 (Aug., 1936), *Univ. Calif. Pub. Bot.*, 19, No. 6 (May, 1937) and *Proc. Nat. Acad. Sci.*, 23, 169–174 (May, 1937)), we felt that we had made progress in the way of distinguishing the various species of *Iridaea* from one another and also we found it desirable to assign a new name, *Iridophycus*, to the genus in order to avoid the confusion attending the earlier *Iridea* of Stackhouse (1816) as well as the confusion attending the designation of *I. edulis* (Stackh.) Grev. (1830) and *I. cordata* (Turn.) Bory by J. G. Agardh (1847) as types of the genus. Also, in order to avoid confusion, we designated *I. capensis* J. Ag. as the type under the new generic name, since type specimens of this species were in existence and no type specimen of *Fucus cordatus* was known.

The Hooker Herbarium at Kew, after exhaustive searching both by myself and by the staff at Kew, failed to reveal any specimen which could be regarded as the type of *Fucus cordatus* Turner. The collections at the British Museum of Natural History likewise were searched to no avail and Gardner and I seemed justified in assuming that no type had been saved since Dawson Turner, himself, saved no types, according to Sir Joseph Dalton Hooker, his grandson, who states in a letter preserved at Kew: "My grandfather was not the sort of man to collect and preserve plants" (*Proc. Linn. Soc. London*, 150th session, pt. 1, 22 (Dec, 1937)). We may presume that such of Dawson Turner's Algae as are to be found in the "Hooker Herbarium" were preserved through the meticulous care of his son-in-law, Sir William Jackson Hooker, who is responsible for so many of the excellent drawings of Turner's "Fuci."

While there seemed to be no doubt from our previous studies that *Fucus cordatus* Turn. was not among the *Iridophycus* species known from the Southern Hemisphere, the question as to its exact characters became acute as Gardner and myself attempted to unravel the complex of the species of the coasts of western North America and northeastern Asia. Following out a suggestion of one of our fellow botanists (Dr. Ivan M. Johnston of the Gray Herbarium) that some of the plants collected by Archibald Menzies were to be found at the Royal Botanic Gardens at Edinburgh, a letter was addressed to the Regius Keeper, Sir William Wright Smith, with the result that a sheet of two specimens of "*Fucus cordatus*" collected by "A. M., Banks' Isles, N. W. Coasts of America" were found in the "Menzies Herbarium" and generously forwarded to us for study. The lower specimen answers so very closely to Turner's figure of the plant (*Fuci*, 2, pl. 118) that it seems possible that it may be the very specimen from which the drawing was made. It is, moreover, just one-half the diameter of the figure of Turner, who says, however, that it "contracts to little more than half its original size" in drying. It seems, therefore, since in all its characters it corresponds to Turner's description, that here we have the type material, or if any doubt remains, at least duplicate type

material, and it is possible to study the cystocarpic type in all essential detail.

*Fucus cordatus* Turner, both as described by Turner and as represented by the Menzies specimens in Herb. Edinb. is a plant of the size of the series of larger species of *Iridophycus* (cf. Setchell and Gardner, *Proc. Nat. Acad. Sci.*, 23, 174 (Mar., 1937)). Turner describes it as about a foot long and 6 inches wide, which corresponds to the larger of the two specimens in the Menzies Herbarium, after allowing about half diameter for shrinkage during drying. The fronds, according to Turner, arise from "a largish, expanded, callous disk" and "the fronds are numerous from the same base." There is a distinct stipe, about 1.2 cm. long ("6 lines," according to Turner) which is flattened, narrow below and expanding above into a narrow cuneate apophysis, distinctly set off from the stipe below and the broad flaring base of the lamina (or blade) above and about 1 cm. long and 0.6–1 cm. broad. The color of the whole plant from basal disc to apex is described by Turner as "livid-brown, not without a tinge of purple, transparent, darker when dry, and then looking almost black unless held up to the light," with "substance" described as between "coriaceous and fleshy, thick and full of moisture."

The blade is broadly ovate-oblong, with broad, shallow, cordate base, more or less unsymmetrical, narrowing in the upper third to a broad and obtuse point. The margins are devoid of projections or lobes, "quite entire" is Turner's description, but are broadly undulate, with the very small cystocarps of extraordinarily uniform size, scattered over the entire blade with the exception of a crescentic zone just above the apophysis. Turner says: "in the lower and narrowed part of the frond the margins are remarkably elevated and incurved and this part consequently looks not only like a stem, but a channeled one." This shows also in the Menzies specimens and indicates that the species has a very definite apophysis set off from both the blade above and the short stipe below.

The cystocarps are described by Turner (loc. cit., p. 118) as being "spherical tubercles, smaller than a pin's head, of a tawny color, scattered without order all over the frond, and immersed in the middle of its substance, containing a number of minute, oblong, red seeds, together with pellucid jointed fibers." While the general morphology of *Fucus cordatus* is almost sufficient to stamp it as a very well defined species among those of *Iridophycus*, the uniformity of the cystocarps together with their comparatively small size, add to the other indications that it is most distinct and, as will be indicated later, evidently restricted to a small region in northwestern North America.

Transverse sections of the plant of Menzies are from about 186 to 245  $\mu$  thick in the sterile portions (when only moderately swollen up, to about what seems normal when fresh (i.e., cut by freezing method in 70 parts

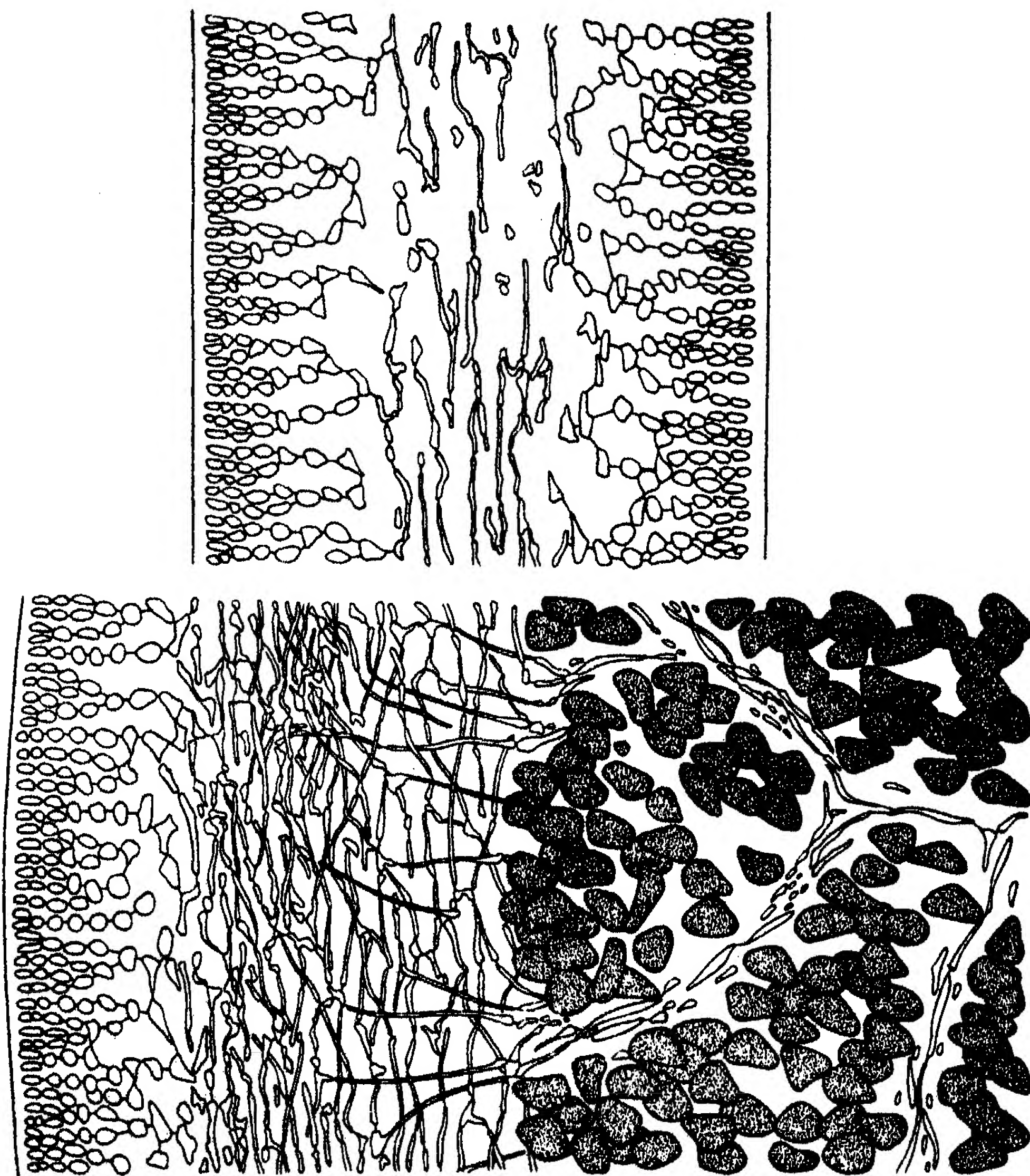
of glacial acetic acid and 30 parts of water, mounted in "Karo") while the cystocarpic portions are up to  $375\ \mu$  thick or even somewhat thicker. That portion of the blade (see figure 1) which is not cystocarp-bearing shows a medullary layer occupying about three-sevenths of the diameter of the section and consisting in very thin section of about 4 more or less parallel slender and loosely placed filaments, about  $3\text{--}4\ \mu$  diam. The segments of the hyphae are commonly elongated (up to  $28\ \mu$  long). In thin sections cut about  $15\ \mu$  thick and treated so as to prevent undue swelling, the filaments of the medulla show practically parallel (as in figure 1), but in thick sections (or crushed fragments) the hyphal segments form an irregular but close reticulum often with elongated meshes and with many free ends, enough to disturb the regularity of the reticulum. In thicker or more swollen sections the irregular network is definitely apparent but never approaches the regular reticulum of the *Iridaea cordata* of Kuetzing (*Phyc. Gen.*, 395, pl. 77, II (1843), *Tab. Phyc.*, 17, pl. 6, fig. b (1867)) from Valparaiso, which is obviously not Turner's species but *Iridophycus Boryanum* Setch. et Gard. (*Proc. Nat. Acad. Sci.*, 22, 470 (1936)).

The cortex of the sterile portions of the frond is indistinctly double (as in most species of the genus), divided into an outer and an inner. The outer cortex consists of closely packed anticlinal rows (or anticlinal groups) of cells, 6–8 in number (vertical to the surface). These cells form a perfectly distinct layer of about 6 cells from the surface, the lower larger and rounded or slightly flattened, the upper 3 (or 4) slightly elongated vertical to the surface. The outermost, commonly single, may be globular to cartridge-shaped. Occasionally the outermost cells arise by twos from the cell below and are decidedly elongated as indicated by Kylin (*Lund Univ. Årsskr.*, n. f., Avd. 2, 24, 47, fig. 25 (1928)).

The lower portion of the outer cortex is not, in cystocarpic and antheridial plants, sharply delimited, but between it and the medulla there is a tissue, undefined on outer and inner sides, of several layers of distant, horizontally elongated cells, short to fairly long in horizontal diameter (parallel to the surface of the frond) which exists in all species of *Iridophycus* and which, because of its augmentation in the tetrasporic plants of the genus, may be distinguished as an inner cortex. This undefined inner cortex equals approximately the thickness of the outer cortex. The comparatively slight development in the inner cortex in sterile, antheridial and cystocarpic individuals of species of *Iridophycus* as compared with tetrasporic individuals makes for the extreme increase in thickness found in the last and is to be emphasized here in the case of the type of *Fucus cordatus* since search for the proper tetrasporic type is utterly complicated as to thickness and complexity of structure.

The cystocarps in Turner's plant (cf. figure 2) measure up to about  $500\ \mu$





FIGURES 1 AND 2

*Iridophycus cordatum* (Turn.) S. et G.

Fig. 1 (above). Portion of a transverse section of a sterile portion of the blade of the "type" specimen.  $\times 400$  diam.

Fig. 2 (below). Portion of a similar section showing the upper segment of a cystocarp  $\times 400$ .

(Drawn by Roy W. Donley under direction of the author)

in horizontal diameter and about  $315\ \mu$  in vertical diameter. The spores are in definite groups, separated by slender "nutritive" filaments, and the whole cystocarp is enclosed within a distinct and fairly thick ( $60\text{--}65\ \mu$ ) layer of concentrically disposed filaments, the "*pericarpium proprium*," from whose filaments radiating "nutritive" filaments pass into the groups of spores (from gonimoblastic lobes) and join the nutritive filaments surrounding each of them (see figure 2). The *pericarpium proprium* arises from the inner cortex. The cystocarps form rather to one surface or the other of the frond, enlarging to become almost central at maturity and causing the surfaces of the frond to bulge out slightly and almost equally on both surfaces. The large, densely filled auxiliary cells ("Tragzellen") are shown in many of the immature cystocarps of the sections from the type material and in very characteristic fashion (cf. Kylin, "Anat. d. Rhodophyc.," figure 210A (1937)).

The cystocarpic type of *Iridophycus cordatum* (Turner) Setchell et Gardner (*Proc. Nat. Acad. Sci.*, 23, 170 (Mar., 1937)) seems clearly established from the Menzies specimens in the Herbarium of the Royal Botanic Garden of Edinburgh. Its salient points are the distinct stipe, equally distinct broadly cuneate apophysis, the flaring, more or less shallow cordate base of the ovate blade with smooth but undulate margins, and the regularly and thickly scattered small cystocarps of uniform and small diameter. Added to this, the blade is only moderately thick and of a brownish purple color, becoming opaque and blackish on drying. Examining sections of dried material (cut by the freezing method in 70 parts of glacial acetic acid to 30 of water and mounted in "Karo," so as to prevent undue swelling), the medullary layer shows 3–4 (?) moderately slender filaments with usually a parallel course although anastomosing into an elongated and irregular reticulum with many free endings as seen in the thicker sections or on crushing. If only a little more water is added the sections enlarge very considerably and even become disorganized, as is the case with other species of the genus and even with most Gigartineae. The cortices are also reasonably distinctive, consisting of a few layers of larger, widely separated cells of the inner cortex and an outer cortex of about 6 cells in each anticlinal row. The cystocarpic portions of the fronds bulge slightly on both surfaces and the flattened, bluntly lenticular cystocarp has a distinct *pericarpium proprium*.

Studies of various plants, particularly those collected from the general Puget Sound region and northward, for occurrences of *Fucus cordatus* have been made with the following results: (1) no specimens from south of Cape Flattery; (2) a number of specimens from the west coast of Whidbey Island, Wash., collected by N. L. Gardner, which agree within fair limits; (3) some young specimens from Table and Calvert Islands, B. C., collected by T. T. and E. E. McCabe, which are in the general distribu-



tional area of Banks Island, B. C.; and (4) two specimens collected by John Macoun on Vancouver Island, B. C., without special data, but judging from Macoun's correspondence, probably in the neighborhood of Ucluelet on the west coast area about May, 1909. None of these various specimens is mature cystocarpic, but several are young or mature tetrasporic, while some may possibly be young antheridial. The lack of additional mature cystocarpic specimens (the type specimen being cystocarpic) for exact comparison and since tetrasporic plants of species of *Iridophycus* differ from cystocarpic especially in details of thickness and difference of histologic details due to the usually prominent tetrasporogenic layer, make the lack of any additional specimens from the exact type locality unfortunate.

Since both cystocarpic and tetrasporic plants are necessary to understand a species, it has seemed reasonable to assume that the tetrasporic generation is represented by such plants as were included by N. L. Gardner under his No. 58, cast ashore on the western shores of Whidbey Island, Wash. (Herb. Univ. Calif. Nos. 464012, 547647, 547648). Another mature tetrasporic plant is No. 72 of John Macoun, probably collected at or near Ucluelet, on the west or ocean side of Vancouver Island in 1909. All of these plants agree in general habit with the type specimen (cystocarpic) but differ in minor details of gross and naturally, of microscopic anatomy. They vary up to about twice the length of the type and in one at least the blade is very broad. The blade varies from a moderate flare above the apophysis to shallow cordate, while the tip is shallowly to fairly deeply and broadly lobed into two to three divisions. None is cleft to (or through) the apophysis. The color of the mature fruited plants is deep claret to almost black in the dried specimens.

The stipe is comparatively long (1.2-1.5 cm.) and about 1.5 mm. thick, cylindrical at the very base, compressed above. The apophysis is distinct, broad cuneate, 1-2 cm. long and about 2 cm. broad above. The blade flares suddenly and with a shallow cordate to reniform base. A similar habit, to a certain degree at least, is to be found in *I. coriaceum* S. et G., *I. splendens* S. et G., *I. lineare* S. et G., *I. whidbeyanum* S. et G., *I. fulgens* S. et G. and *I. sinicola* S. et G., but the coarse, crowded, short and irregular segments of the medulla of the blades of these species easily distinguish them, as well as minor differences in stipe, apophysis and blade. The nearest approach to *I. cordatum* (Turn.) S. et G. is *I. flaccidum* S. et G., but there are differences in color, stipe, apophysis, thickness and medullary structure sufficient to separate characteristic plants of this species although, at times, there may be difficulty in placing young or sterile specimens.

Tetrasori in the plants selected as typical (N. L. Gardner No. 58, as indicated above) are very thickly and regularly scattered over the whole

blade (at maturity) except that portion of it immediately above the apophysis. In more translucent dried specimens they appear as fairly uniform, small, dark, circular to elongated-oblong patches, on both surfaces, each of which is slightly elevated above such sori as occur directly beneath it. The mature sori are separated from one another by intervals less than that of their own diameters. In cross-section the tetrasporic plants show the same type and thickness of medulla as found in the cystocarp type plant, but differ, as do all the tetrasporic plants of the genus *Iridophycus*, in details of the outer and inner cortices. The outer cortex of the tetrasporic plant differs from that of the cystocarpic plant in having the terminal cells of the anticlinal rows more regularly in pairs and somewhat to very considerably elongated, a matter of only occasional occurrence in cystocarpic plants, and giving their cortices a decidedly different aspect. The most modifying difference between cystocarpic and tetrasporic plants, however, is in the voluminous development of the tissue between the medulla (proper) and the outer cortex (proper), since as in all the species of *Iridophycus*, this "inner cortex," not very distinctly set off from the "outer cortex" in the sterile, antheridial and cystocarpic plants, increases in thickness, and through the multiplication of cells forming a complex of elongated and connecting filaments between the medullary segments on the one side and the anticlinal rows of the cortex on the other, a definite sporogenic tissue is formed up to about 100  $\mu$  to 150  $\mu$  in thickness (on each side). In this tetrasporogenic plexus, the intercalary cells enlarge, multiply, pull away from one another and form finally the tetrasporangial mother cells, from which by division at right angles the tetraspores are formed. The tetrasporangia are thus intercalary or interstitial as to origin, but are "accessory" in the sense that they occur as a multiplication of cells of the inner cortex (not of the outer cortex or of the medulla) into a distinct tetrasporogenic tissue.

As may be understood from the statement of the intercalation of a distinct and relatively thick tetrasporogenic tissue on each side of the medulla, the thickness of a fertile (or even immature) tetrasporic plant will greatly exceed that of the sterile portions of the cystocarpic plant. While the sterile portions of a tetrasporic plant may be little over 250  $\mu$  as in the cystocarpic plant, the thickness of the greater part of a tetrasporic plant (according to stages toward maturity) may increase up to 685  $\mu$  or less. In such a section the medulla accounts for about 63  $\mu$  of the thickness and each of the outer cortices for about the same, while each tetrasporogenic tissue, in sterile portions of the blade only about 20–25  $\mu$  as an inner cortex, has become 150–200  $\mu$  thick, according to its maturity. The tetrasporogenic layers are not absolutely continuous, but slight breaks may occur in them.

The sori are distinct, flattened spheroid, usually about 60–65  $\mu$  in

vertical diameter at maturity and vary in their horizontal diameter from about the same up to  $625\ \mu$  and (exceptionally) even up to  $1000\ \mu$ . They impinge upon the true medulla inwardly and upon the outer cortex outwardly, and cause bulges, above each, of the outer cortex. The tetrasporangia are somewhat variable in size, elongated ellipsoid, about  $28 \times 14\ \mu$  divided cruciately into the 4 tetraspores.

*Summary.*—It has seemed best to consider the *Fucus cordatus* Turn. in sufficient detail to make clear its nature, since it has been regarded as:

1. The morphological and even the taxonomic type of the genus *Iridaea* of Bory;
2. The species of widest distribution through Pacific North and South America and even South Africa;
3. A species whose type specimen had been lost, until the discovery of the Menzies specimen in Herb. Edinburgh.

With the discovery of that type and the realization that there were somewhere about 30 species of *Iridophycus* segregated in at least 4 distinct sections (or subgenera?), the necessity for some exact knowledge of the nature and distribution of *Fucus cordatus* Turn. became imperative. There results from this study, not only of the cystocarpic type specimen but also of the tetrasporic plants seemingly undoubtedly to be referred to it, the following:

1. That it has a distinct, relatively long stipe;
2. That its apophysis is broad cuneiform, distinctly set off from both the stipe below and the blade above;
3. That the blade is rather broadly cordate, with some variation as to flare at the base;
4. That the tip is broadly and obtusely pointed, either entire or 2-3-lobed;
5. That it belongs to the group of larger species of *Iridophycus*;
6. That its medullary tissue is of slender filaments, anastomosing into an irregular reticulum, and to be distinguished by this from species of similar aspect whose medullary filaments are coarse, short articulate and not forming apparent reticula;
7. That the tetrasporogenic inner cortex is highly developed in the tetrasporic plant, as in other species of *Iridophycus* where this tissue is continuous;
8. That both the mature and the immature cystocarps are typical of *Iridophycus*;
9. That its distribution is most probably confined to the shores of north-western North America, not having been found south of the Straits of Juan de Fuca, and certainly bearing no relation to the South American or South African species earlier considered identical with it.

## EVERY TWO EQUAL ORDER SUBGROUPS HAVING ONLY IDENTITY IN COMMON

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The term subgroup will be used in the present article for a proper subgroup, that is, for a subgroup which is neither the identity nor the entire group. Suppose that a given abelian group  $G$  has a set of subgroups of the common order  $p^k$ ,  $p$  being a prime number, such that every pair of these subgroups has only the identity in common and that all the operators of  $G$  appear in these subgroups. The order of  $G$  is then of the form  $p^m$ . To prove that  $m$  is divisible by  $k$  it may first be noted that such a  $G$  contains an invariant subgroup of order  $p^k$  and that if this subgroup is extended by another such subgroup contained in  $G$  there results an invariant subgroup of order  $p^{2k}$  when  $m > 2k$ . Similarly there appears in  $G$  an invariant subgroup of order  $p^{3k}$  whenever  $m > 3k$ , etc. Hence there results the theorem that *whenever a group contains a set of subgroups of order  $p^k$ ,  $p$  being a prime number, such that all the operators of the group appear in these subgroups and every two of them have only the identity in common, then its order is  $p^m$  and  $m = nk$ , where  $n$  and  $k$  are positive integers.*

The simplest case presents itself when all the operators of  $G$  besides the identity are of order  $p$  irrespective of whether  $G$  is abelian or non-abelian. The number of the subgroups of order  $p$  in such a  $G$  is  $(p^m - 1)(p - 1)$ . We proceed to prove that whenever  $G$  is an abelian group of order  $p^m$  and of type  $1^m$  it is always possible to find in  $G$  a set of subgroups of the common order  $p^k$  such that every operator of  $G$  besides the identity appears in one and only one of these subgroups whenever  $m = kn$  where  $k$  and  $n$  are positive integers. It was proved above that this is a necessary condition. To prove that it is also sufficient we may represent  $G$  as the direct product of  $n$  regular permutation groups of order  $p^k$  and of type  $1^k$  and note the number of the subgroups of order  $p^k$  contained in this direct product which satisfy the condition that every pair of these subgroups has only the identity in common. It is clear that there are  $n$  such regular subgroups and that these involve all the permutations of degree  $p^k$  contained in  $G$ .

The permutations of degree  $2p^k$ , which appears in such a  $G$ , are found in the simple isomorphisms between sets of two such regular groups. In the particular case when  $n = 2$  we thus obtain  $1 + p^k$  subgroups which have only the identity in common since the group of isomorphisms of the abelian group of order  $p^k$  and of type  $1^k$  involves a cyclic group of order  $p^k - 1$ . The number of the subgroups of degree  $np^k$  and of order  $p^k$  in



which all the permutations besides the identity are of degree  $np^k$  is  $(p^k - 1)^{n-1}$  since these may be obtained by establishing simple isomorphisms between the given  $n$  regular group of order  $p^k$ . By establishing simple isomorphisms between the  $n$  sets of  $n - 1$  simply isomorphic regular groups of order  $p^k$  there result  $n(p^k - 1)^{n-2}$  groups which have only the identity in common with each other and with the given  $(p^k - 1)^{n-1}$  groups of degree  $np^k$ , etc. Hence the total number of the subgroups which have separately only the identity in common and involve all the permutations of  $G$  is

$$p^{(n-1)k} + p^{(n-2)k} + \dots + 1 = (p^{nk} - 1)/(p^k - 1).$$

It is easy to prove that when  $G$  is an abelian group of order  $p^m$  which involves operators whose orders exceed  $p$  then it is impossible to find in  $G$  a set of subgroups of order  $p^k$ ,  $k > 1$ , such that every pair of these subgroups has only the identity in common and that these subgroups involve all the operators of  $G$ . This follows from the fact that such a group contains operators of order  $p^2$  and that all the products of one of its operators of order  $p^2$  into an operator of order  $p$  generate the same subgroup of order  $p$ . If a subgroup which involves a cyclic subgroup of order  $p^2$  has only the identity in common with every other subgroup of the set to which it belongs it therefore involves all the operators obtained by multiplying this cyclic subgroup of order  $p^2$  by all the operators of order  $p$  contained in the group. Hence such a subgroup involves all the operators of order  $p$  contained in the group. As every other subgroup of the set involves operators of order  $p$  and has only the identity in common with this subgroup we have arrived at a contradiction by assuming that in an abelian group of order  $p^m$  which involves operators of order  $p^2$  it is possible to find a set of subgroups involving all the operators of the group and satisfying the condition that every pair of subgroups has only the identity in common.

If the order of an abelian group  $G$  is divisible by at least two distinct prime numbers it is not possible to find therein a set of subgroups of the same order which involve all the operators of  $G$  but involve no two subgroups which have more than the identity in common. If such a set would exist in  $G$  each of the subgroups of the set would involve a cyclic subgroup whose order would be divisible by all the prime numbers which divide the order of  $G$  and therefore by the product of all these prime numbers. If  $p$  is such a prime number no other subgroup of the set could involve an operator of order  $p$ . Hence we have arrived at a contradiction by assuming that the order of  $G$  is divisible by two distinct prime numbers and it has been proved that *the only abelian groups which satisfy the condition that they involve a set of subgroups of the same composite order which include all the operators of the group but include no two subgroups which have more*

than the identity in common are the abelian groups of order  $p^m$  and of type  $1^m$ ,  $p$  being a prime number.

We proceed to prove that every Sylow subgroup of each one of the subgroups of equal order which have no common operator besides the identity and include all the operators of  $G$  is abelian and of type  $1^k$ . It may first be noted that if such a subgroup were non-abelian it would be transformed into itself by an operator in the corresponding Sylow subgroup of  $G$  which does not appear in this subgroup in view of the theorem that in a prime power group every proper subgroup is transformed into itself by an operator of the group which does not appear in this subgroup. As this transforming operator would also appear in one of the set of subgroups which involve all the operators of  $G$  but contain no common operator besides the identity it follows that the latter subgroup would have an operator besides the identity in common with the former which is contrary to the hypothesis. It therefore results that *all the operators of  $G$  which are powers of the same prime number and appear in the subgroups involving no common operator besides the identity but involving all the operators of  $G$  are of prime order.*

Suppose that  $G$  is a non-abelian group of order  $p^m$ ,  $p > 2$ , which involves no operator of order  $p^2$  but contains an abelian subgroup of index  $p$  and a commutator subgroup of order  $p$ . The central of  $G$  is then of order  $p^{m-2}$ . To prove that not all the operators of this  $G$  can appear in subgroups of order  $p^2$  such that every two of these subgroups have only the identity in common it may be noted that if a subgroup of order  $p^2$  is in a subgroup of index  $p$  under  $G$  which includes the central but is not itself in this central then it has just  $p$  operators in common with this central. The number of such possible subgroups is therefore  $(p^{m-2} - 1)/(p - 1)$  and the total number of the operators of  $G$  which appear in these subgroups constitute a subgroup of index  $p$  under  $G$ . It has therefore been proved that in a non-abelian group of order  $p^m$  which involves no operator of order  $p^2$  but has an abelian subgroup of index  $p$  and a commutator subgroup of order  $p$  it is not possible to distribute all the operators into subgroups of order  $p^2$  such that every pair of these subgroups has only the identity in common.

It is not difficult to extend this result so as to apply to abelian subgroups of any larger order contained in such a  $G$ . If the order of such an abelian subgroup would be  $p^k$  it results that  $m$  would be  $nk$  for the same reason as when  $G$  was assumed to be abelian. The operators of such a subgroup which would appear in the central of  $G$  would constitute a group of order  $p^{k-1}$  just as in the case when  $k = 2$ . Hence all the operators of the central would be exhausted by the subgroups found in a subgroup of index  $p$  under  $G$ . It would therefore not be possible to construct the remaining subgroups of order  $p^k$  so that all the operators of  $G$  would appear in these



subgroups and every two of these subgroups would leave only the identity in common.

It can now be easily proved that the Sylow subgroups of  $G$  are abelian and of type  $1^k$ . It was proved above that all the operators of such a group, besides the identity, are of prime order. If the group were non-abelian its operators would appear in sets of conjugate subgroups having only the identity in common since  $G$  could not be a Hamiltonian group. These subgroups would be transformed according to a transitive permutation group under this Sylow subgroup. The subgroups which correspond to the letters of this permutation group could not all be transformed into different such subgroups by any one of these subgroups. They would therefore be thus transformed by an operator of this Sylow subgroup which is found in the other such subgroup since every transitive permutation group involves permutations which permute all the letters of the group.

This operator would transform this set of conjugate subgroups among themselves and hence it would transform the non-invariant operators of the set into operators of the set. The subgroup of the given common order of distinct subgroups which involve all the operators of the given Sylow subgroup to which the operator in question belongs would therefore be transformed into itself multiplied by an operator found in the given conjugate set of subgroups. As this is impossible it results that the Sylow subgroup in question is abelian and hence there results the following theorem: *If a non-abelian group has the property that all of its operators appear in a set of subgroups of the same order such that every two of these subgroups have only the identity in common then the Sylow subgroups of this group are abelian and of type  $1^k$ .*

## GENERALIZED HOMOLOGY GROUPS

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We propose the following generalization of homology groups:

In a topological space  $M$  we define an unoriented *singular* simplex in the usual manner as a continuous map of a euclidean simplex onto  $M$  (Seifert-Threlfall, p. 92). As the coefficient group of the chains corresponding to the singular simplexes, we choose the group of all integers modulo a prime integer  $p$ .

We define the *boundary* of a singular simplex  $A^i$  ( $i > 0$ ) to be the chain consisting of the  $(i - 1)$ -dimensional (singular) faces of  $A^i$  where each

face is taken with the coefficient *plus one*. This definition is equivalent to the usual one only for the case  $p = 2$ . The case  $p = 3$  is discussed below. The boundary  $B(K^i)$  of a chain  $K^i$  is the sum, modulo 3, of the boundaries of the constituent simplexes of  $K^i$ .

It is easy to verify that (for  $p = 3$ ) the third boundary of every chain vanishes (and not the second boundary as in the case  $p = 2$ ), i.e.,

$$B\{B[B(K^i)]\} = 0. \quad (1)$$

Hence, two kinds of "cycles" exist, namely the cycles  $C^i$  of type  $\alpha$  whose first boundaries vanish and the cycles  $C^i$  of type  $\beta$  whose second boundaries vanish. Of course, a cycle of type  $\alpha$  is also of type  $\beta$  (a 1-chain is, by definition, a cycle of type  $\beta$ , a 0-chain, a cycle of type  $\alpha$ ). According to equation (1), the chain  $B[B(K)^{i+2}]$  is an  $i$ -cycle of type  $\alpha$  and  $B(K^{i+1})$  an  $i$ -cycle of type  $\beta$ .

For the case  $p = 3$  two generalizations of the homology groups present themselves for study (and for the case of an arbitrary integer  $p$ ,  $p - 1$  generalizations). The first homology group of dimension  $i$  is the group of all  $i$ -cycles of type  $\alpha$  modulo the subgroup formed by the cycles of type  $\alpha$  that are second boundaries of  $(i + 2)$ -chains: namely, that of the form  $B[B(K^{i+2})]$ . The second homology group is the group of all  $i$ -cycles of type  $\beta$  modulo the subgroup formed by the cycles of type  $\beta$  that are first boundaries of  $(i + 1)$ -chains:  $B(K^{i+1})$ . Obviously the groups here defined are topological invariants.

We have proved that if the space  $M$  is a complex of simplexes the homology groups of  $M$  can be derived by using only the chains determined by the simplexes of the complex (provided we always include "degenerate" simplexes with repeated vertices). For the case  $p = 3$ , these groups remain unchanged if we use only simplexes with vertices of multiplicity no greater than 2. In other words, a simplex with a vertex of multiplicity of 3 or more plays the same rôle (for the case  $p = 3$ ) as a degenerate simplex in the classical theory. That is to say, all simplexes of this sort may be identified with the chain 0 without altering the homology groups.

This is the main result so far obtained. A detailed exposition will shortly be given in another journal. The proofs, as in the case of the ordinary homology groups, are based on the fundamental idea of a simplicial approximation.

# FUNCTIONS WHOSE EVEN DERIVATIVES HAVE A PRESCRIBED SIGN

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R. P. Boas has suggested to the author that one might hope to use Lidstone series to prove the analyticity of a function whose even derivatives do not change sign on a given interval. The original idea was to prove that if these even derivatives are all positive then the function is analytic on the interval in question. The method turned out to be of no use for this particular problem, but Boas<sup>1</sup> gave a proof of the result independently by another method. However, his suggestion led the author to prove the wholly unexpected result that if

$$(-1)^k f^{(2k)}(x) \geq 0 \quad (k = 0, 1, 2, \dots) \quad (1)$$

on any interval, however small, then  $f(x)$  is entire. The functions  $\sin x$  and  $\cos x$  are illustrative examples.

In this note we give a complete proof of the above result, using a minimum of the theory of Lidstone series. In a later paper we shall go into the relation of the present result to the problem of the representation of functions by such series.

The Lidstone series for a given function  $f(x)$  is defined as

$$\sum_{n=0}^{\infty} f(1) \Lambda_n(x) + f(0) \Lambda_n(1-x),$$

where

$$\Lambda_0(x) = x$$

$$\Lambda_n^*(x) = \Lambda_{n-1}(x)$$

$$\Lambda_n(0) = \Lambda_n(1) = 0 \quad (n = 1, 2, \dots).$$

These conditions determine the polynomials  $\Lambda_n(x)$  completely.

Define the functions  $G_n(x, t)$  as follows:

$$\begin{aligned} G_1(x, t) &= (t-1)x \quad (t > x) \\ &= (x-1)t \quad (t < x) \end{aligned}$$

$$G_n(x, t) = \int_0^1 G_1(x, u) G_{n-1}(u, t) du \quad (n = 2, 3, \dots).$$

It is easily seen that

$$\Lambda_n(x) = \int_0^1 G_n(x, t) t dt.$$

With these preliminaries we now prove our result by a series of theorems.

**THEOREM 1.** *If  $f(x) \in C^{2k}$  on  $(0 \leq x \leq 1)$ , then*

$$f(x) = \sum_{n=0}^{k-1} f^{(2n)}(1) \Lambda_n(x) + f^{(2k)}(0) \Lambda_k(1-x) + R_k(x) \quad (2)$$

$$R_k(x) = \int_0^1 G_k(x, t) f^{(2k)}(t) dt. \quad (3)$$

This is proved by integrating the integral (3) by parts.

Now observe that for any positive integer  $k$

$$(-1)^k G_k(x, t) \geq 0 \quad (0 \leq x \leq 1, 0 \leq t \leq 1),$$

so that if (1) holds on  $(0 \leq x \leq 1)$ , then all the terms of (2) are non-negative. This is the essential fact which enables us to obtain bounds on the successive derivatives of  $f(x)$ .

**THEOREM 2.** *For any positive integer  $k$*

$$(-1)^k \Lambda_k(x) \geq \frac{x^{k+1}(1-x)^{k+1}}{(k+2)!} \quad (0 \leq x \leq 1). \quad (4)$$

Observe first that

$$(-1)^k \int_0^1 [G_1(x, t)]^k dt = \frac{x^k(1-x)^k}{k+1}.$$

and that

$$-G_1(x, t) \leq t(1-t) \leq t \quad (0 \leq x \leq 1, 0 \leq t \leq 1).$$

Hence

$$-\Lambda_1(x) = -\int_0^1 G_1(x, t) t dt \geq \int_0^1 [G_1(x, t)]^2 dt = \frac{x^2(1-x)^2}{3} \geq \frac{x^2(1-x)^2}{3!}.$$

That is, (4) is true for  $k = 1$ . Now assume it true for  $k = n - 1$ . Then

$$\begin{aligned} (-1)^n \Lambda_n(x) &= (-1)^n \int_0^1 G_n(x, t) t dt \\ &= (-1)^n \int_0^1 G_1(x, u) du \int_0^1 G_{n-1}(u, t) t dt \\ &= (-1)^n \int_0^1 G_1(x, u) \Lambda_{n-1}(u) du \geq -\int_0^1 G_1(x, u) \frac{u^n(1-u)^n}{(n+1)!} du \\ &\geq \frac{(-1)^{n+1}}{(n+1)!} \int_0^1 [G_1(x, u)]^{n+1} du = \frac{x^{n+1}(1-x)^{n+1}}{(n+2)!}. \end{aligned}$$

Thus the proof is completed by induction.

**THEOREM 3.** *If (1) holds on  $(0 \leq x \leq 1)$ , then*

$$f^{(2k)}(1) = O[4^{k+1}(k+2)!] \quad (k \rightarrow \infty). \quad (5)$$

For, from (1) and (2) we have

$$0 \leq f^{(2k)}(1)\Lambda_k(x) \leq f(x) \quad (0 \leq x \leq 1).$$

Setting  $x = 1/2$  and making use of (4) we have (5).

**THEOREM 4.** *If (1) holds on  $(0 \leq x \leq 1)$ , then there is a constant  $M$  such that*

$$(-1)^k f^{(2k)}(x) \leq M4^{k+1}(k+2)!/x^{2k} \quad (0 < x \leq 1). \quad (6)$$

For, if  $f(x)$  satisfies (1) on an interval  $(0 \leq x \leq b)$ , then  $F(x) = f(bx)$  does so also on the interval  $(0 \leq x \leq 1)$ . Applying Theorem 3 to  $F(x)$  we have

$$F^{(2k)}(1) = b^{2k} f^{(2k)}(b) = O(4^{k+1}(k+2)!). \quad (7)$$

Replacing  $b$  by  $x$  in (7) gives (6).

**THEOREM 5.** *If (1) holds on  $(0 \leq x \leq 1)$ , then  $f(x)$  is entire.*

For, from (6) we see that

$$f^{(2k)}(x) = O(4^{k+1}(k+2)!) \quad (k \rightarrow \infty) \quad (8)$$

uniformly in  $(a \leq x \leq 1)$ , where  $a$  is any positive number less than unity. By a familiar relation<sup>2</sup> between the bounds of the even derivatives and the bounds of the odd derivatives we have

$$f^{(2k+1)}(x) = O(4^{k+2}(k+3)!) \quad (k \rightarrow \infty) \quad (9)$$

uniformly in  $(a \leq x \leq 1)$ . Relations (8) and (9) are sufficient to show that the Taylor expansion of  $f(x)$  converges to  $f(x)$  for all  $x$ .

This result has the following interesting consequence.

**THEOREM 6.** *If*

$$f(x) = \sum_{n=0}^{\infty} a_n x^n,$$

*the coefficients  $a_n$  being real and such that*

$$(-1)^n a_{2n} > 0 \quad (n = 0, 1, 2, \dots)$$

$$\lim_{n \rightarrow \infty} \sqrt[n]{|a_n|} > 0,$$

*then in any interval  $(0 \leq x \leq \delta)$ , however small, some even derivative of  $f(x)$  changes sign.*

<sup>1</sup> This proof will appear in an early issue of the *Duke Mathematical Journal*.

<sup>2</sup> See, for example, T. Carleman, *Fonctions Quasi Analytiques*, Paris (1926), p. 12.

## ON GREEN'S FORMULA

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Green's formula can be derived in the following way: For rectangular domains as the limit of an identity, for more complicated domains by virtue of elementary continuity properties of the integrals.

1. *Rectangular Domains.*—Let  $u(x, y)$  be a function defined in the rectangle  $R(a \leq x \leq A, b \leq y \leq B)$ . If  $a = x_0 < x_1 < \dots < x_m = A$  and  $b = y_0 < y_1 < \dots < y_n = B$ , then the following identity may be verified at a glance

$$\sum_{ij} \frac{u(x_i, y_{j+1}) - u(x_i, y_j)}{y_{j+1} - y_j} (x_{i+1} - x_i)(y_{j+1} - y_j) = \sum_i [u(x_i, B) - u(x_i, b)](x_{i+1} - x_i). \quad (\text{I})$$

If  $u_y = \frac{\partial u}{\partial y}$  exists in  $R$ , then by the mean value theorem

$$\frac{u(x_i, y_{j+1}) - u(x_i, y_j)}{y_{j+1} - y_j} = u_y(x_i, \eta_{ij})$$

where  $y_j < \eta_{ij} < y_{j+1}$ . Thus from (I) we get

$$\sum_{ij} u_y(x_i, \eta_{ij})(x_{i+1} - x_i)(y_{j+1} - y_j) = \sum_i [u(x_i, B) - u(x_i, b)](x_{i+1} - x_i). \quad (\text{II})$$

If we assume that the Riemann integrals  $\int \int_R u_y dx dy$  and  $\int_a^A [u(x, B) - u(x, b)] dx$  exist, then in (II) the left side is a Riemann sum for the former, the right side one for the latter integral. By applying (II) to a sequence of decomposition of  $R$  whose norms approach 0, we thus get from (II)

$$\int \int_R u_y dx dy = \int_a^A [u(x, B) - u(x, b)] dx. \quad (\text{III})$$

If we assume that  $\int_a^A u(x, B) dx$  exists, then this integral is =

$-\int_a^A u(x, b) dx$ , and from (III) Green's formula for  $R$  follows:

$$\int \int_R u_y dx dy = - \int_R u(x, y) dy \quad (\text{IV})$$



the integral on the right side being taken on the contour of  $R$  in the counter-clockwise sense.

2. *Remarks and Corollaries.*—Traditionally, Green's formula for simple domains is proved by iterated integration. But in order to apply the formula  $\int \int_R f(x, y) dx dy = \int_a^A dx \int_b^B f(x, y) dy$  to  $f(x, y) = u_y(x, y)$  one has to assume<sup>1</sup> that the function  $F(x) = \int_b^B u_y(x, y) dy$  is integrable in  $[a, A]$  while our proof of (III) does not require such an assumption. By means of Volterra's bounded function which is a derivative without admitting a Riemann integral it is easy to construct a function  $u(x, y)$  for which  $\int \int_R u_y dx dy$  and  $\int_R u dx$  exist (thus our proof works) and yet  $\int_b^B u_y(x, y) dy$ , for some values of  $x$ , does not exist (thus the classical proof does not work).

In the same way as (II) we derive the identity (II')

$$\sum_{ij} u_y(\xi_i, \eta_{ij})(x_{i+1} - x_i)(y_{j+1} - y_j) = \sum_i [u(\xi_i, B) - u(\xi_i, b)](x_{i+1} - x_i)$$

for any choice of  $\xi_i$  between  $x_i$  and  $x_{i+1}$ . If  $S$  is any Riemann sum for  $\int_a^A [u(x, B) - u(x, b)] dx$  of a norm  $< \nu$ , then  $S$  is the right side of (II') for a proper choice of the  $x_i$  and  $\xi_i$ . By (II'),  $S$  is equal to a Riemann sum for  $\int \int_R u_y dx dy$  whose norm is  $< \nu$ . Thus from the existence of  $\int \int_R u_y dx dy$  we conclude:  $\int_a^A [u(x, B) - u(x, b)] dx$  and, in general,  $\int_a^A [u(x, y') - u(x, y'')] dx$  for each  $y'$  and  $y''$ , exists. If  $\int_a^A u(x, B) dx$  exists, then (IV) holds. If  $\int_a^A u(x, y) dx$  exists for *one* value of  $y$ , it exists for *each* value of  $y$ . But  $\int \int_R u_y dx dy$  may exist without  $\int_a^A u(x, y) dx$  existing for any  $y$ . Example:  $u(x, y) = f(x)$ , for each  $y$ , where  $f$  is not Riemann integrable in  $[a, A]$ .

If we *define* the integral  $\int \int_R u_y dx dy$  as the limit of the sums on the left side of (I) which may be called its Weierstrass sums,<sup>2</sup> then this integral exists and is equal to the Riemann integral  $\int \int_R u_y dx dy$  whenever the latter exists. But the Weierstrass integral exists also in many other cases. Moreover, the Weierstrass integral automatically satisfies Green's formula whenever  $\int_a^A [u(x, B) - u(x, b)] dx$  exists, i.e., whenever  $u(x, B) - u(x, b)$  is almost everywhere continuous, without any assumption about the behavior of  $u$  in the interior of  $R$ .

3. *Simple Closed Curves.*—In a closed domain  $D$  let  $u(p) = u(x, y)$  be continuous,  $|u(p)| \leq U$ , and  $|u(p) - u(p')| < \sigma$  whenever the distance between  $p = (x, y)$  and  $p' = (x', y')$  is  $< \delta(\sigma)$ . We set  $\lambda(p, p') = u(p)(x' - x)$  and  $\lambda(\pi) = \sum \lambda(p_i, p_{i+1})$  if  $\pi$  is the ordered set  $\{p_0, p_1, \dots, p_n\}$ . We remark that  $|\lambda(\pi) - \lambda(p_0, p_n)| < \sigma l(\pi)$  if  $\pi$  is contained in a circle of diameter  $\delta(\sigma)$ , and  $l(\pi)$  is the length of  $\pi$ . If  $C$  is a rectifiable curve, then we denote by  $\lambda(C)$  the  $\lim \lambda(\pi)$  for the finite subsets of  $C$ , properly ordered, as they get indefinitely dense in  $C$ ; that is to say,  $\lambda(C) = \int_C u dx$ . We have (1)  $\lambda(-S) = -\lambda(S)$  if  $S$  and  $-S$  are opposite segments; (2)  $\lambda(V) =$

0 if  $V$  is a vertical segment; (3)  $|\lambda(C) - \lambda(S)| < 2\sigma l(C)$  if the curve  $C$ , of length  $l(C)$ , from  $p$  to  $p'$  is contained in a circle of diameter  $\delta(\sigma)$ , and  $S$  is the segment  $pp'$ . For from the previous remark we have  $|\lambda(C) - \lambda(p, p')| < \sigma l(C)$  and  $|\lambda(S) - \lambda(p, p')| < \sigma l(S) \leq \sigma l(C)$ . If in  $D$  the function  $u_y$  exists, is bounded, say,  $|u_y| \leq U_y$ , and admits a Riemann integral  $\kappa$ , then (4)  $|\kappa(A)| \leq U_y a(A)$  for each domain  $A$  of area  $a(A)$  for which  $\kappa(A) = \int \int_A u_y dx dy$  exists.

If  $T = \{p_0, p', p''\}$  is a triangle whose side  $p'p''$  is vertical, then, by vertical segments,  $T$  may be decomposed into a triangle  $T_0$  with the vertex  $p_0$  and trapezoids  $T_1, \dots, T_n$  such that each  $T_i$  is the sum of a rectangle  $R_i$  and two triangles  $T_i'$  and  $T_i''$  (or one if  $T$  has a horizontal side), all oriented in the same sense as  $T$ . In view of (2) and (3) we have  $|\sum \lambda(T_i) - \sum \lambda(R_i)| < \sigma l(T)$  if the slices are so thin that each non-vertical side of the  $T_i$  is  $< \delta(\sigma)$ . Hence  $|\lambda(T) - \sum \lambda(R_i)| < \sigma l(T) + Ul(T_0)$  which can be made as small as we please by choosing  $\sigma$  and  $T_0$  sufficiently small. If, moreover, the slices are so thin that the area of  $T_0 + \sum(T_i' + T_i'')$  is sufficiently small, then, in view of (4),  $|\kappa(T) - \sum \kappa(R_i)|$  is arbitrarily small. Since Green's formula holds for each  $R_i$  it thus holds for  $T$ .

If  $P$  is a simple closed polygon,  $P$  can be decomposed into a finite number of triangles without common interior points, and each of them into at most two triangles with a common vertical side. Since Green's formula holds for each of these triangles, in view of (1) it holds for  $P$ .

If  $C: p(t)$  ( $0 \leq t \leq 1$ ),  $p(0) = p(1)$  is a closed rectifiable curve, we divide  $C$  into parts  $C_i$  between  $p(t_i) = p_i$  and  $p_{i+1}$  ( $0 = t_0 < t_1 < \dots < t_n = 1$ ) so small that each  $C_i$  is contained in a circle of diameter  $\delta(\sigma)$ . By adding the inequalities  $|\lambda(C_i) - \lambda(S_i)| < 2\sigma l(C_i)$  resulting from (3), we get  $|\lambda(C) - \lambda(P)| < 2\sigma l(C)$  where  $P$  is the polygonal line  $\{p_0, p_1, \dots, p_n\}$ . Being rectifiable  $C$  can be covered with a finite number of squares whose sum,  $Q$ , has an arbitrarily small area. By choosing  $P$  so dense in  $C$  that it is contained in a  $Q$  whose area is sufficiently small, we make the area between  $C$  and  $P$ , and hence, by (4),  $|\kappa(C) - \kappa(P)|$  as small as we please.<sup>3</sup> In view of Green's formula for  $P$  we conclude: If the simple closed rectifiable curve  $C$  admits simple closed inscribed polygons, arbitrarily dense in  $C$ , which together with their interior domains are contained in  $D$ , then Green's formula holds for  $C$ . In particular, the assumption is satisfied if  $D$  contains all points common to (1) the smallest convex set containing  $C$ , and (2) some open set containing  $C$  and its interior domain: e.g., if  $C$  and the interior domain of  $C$  are contained in the interior of  $D$ ; or if  $C$  is convex and  $D$  is the domain bounded by  $C$ .

If  $D$  is a domain of the particular form  $[a \leq x \leq A, \varphi(x) \leq y \leq \psi(x)]$  where  $\varphi$  and  $\psi$  are continuous in  $[a, A]$ , then without assuming any continuity properties of  $u$  we can prove by the method of sections 1 and 2

(i.e., by taking the limit of an identity): If in  $D$  the function  $u$ , has a Riemann double integral, then

$$\int_a^A [u(x, \psi(x)) - u(x, \varphi(x))] dx$$

exists and is equal to the double integral. If  $\int_a^A u(x, \omega(x)) dx$  exists for one curve  $y = \omega(x)$  ( $a \leq x \leq A$ ) contained in  $D$ , then the latter integral exists for each such curve. The integrability of  $u$ , thus implies that the function  $u$  (if bounded) is almost everywhere continuous along each curve if it is so along one.

4. *Closed Curves.*—Let  $\rho$  be a point not on the closed curve  $C$ . We denote by  $\alpha(\rho, t)$  the angle which the vector from  $\rho$  to  $p(t)$  includes with the positive  $x$ -axis, and by  $\mu_C(\rho)$  the integer  $[\alpha(\rho, 1) - \alpha(\rho, 0)]/2\pi$ . By a complementary domain of  $C$  we mean a component of the complementary set of  $C$ . For any two points of the same complementary domain  $U$  of  $C$ ,  $\mu_C$  has the same value which we call the multiplicity of  $U$  rel.  $C$ . The unbounded complementary domain of  $C$  has the multiplicity 0 rel.  $C$ .

If  $P$  is a closed polygon with the complementary domains  $U_1, \dots, U_n$  whose multiplicities rel.  $P$  are  $\mu_1, \dots, \mu_n$ , respectively, then  $\lambda(P) = \sum \mu_i \kappa(U_i)$ , where it is sufficient to extend the sum over the complementary domains whose multiplicities rel.  $P$  are  $\neq 0$ . If  $P$  is a simple closed polygon, the statement is Green's formula. In the general case let  $P_i$  be the perimeter of  $U_i$  traversed in the counterclockwise sense. Since each  $P_i$  is a simple closed polygon,  $\sum \mu_i \kappa(U_i) = \sum \mu_i \lambda(P_i)$ . On the other hand,  $\sum \mu_i \lambda(P_i) = \lambda(P)$ . For let  $S$  be a segment of  $P$  common to  $P_i$  and  $P_j$ ,  $\rho_i$  and  $\rho_j$  points of  $U_i$  and  $U_j$  close to the center of  $S$ . Then on  $0 \leq t \leq 1$  the function  $\alpha(\rho_i, t) - \alpha(\rho_j, t)$  increases by  $2\pi$  ( $-2\pi$ ) each time when  $S$  is traversed with  $U_i$  to the left (right), and only then. Thus in traversing  $P$  the algebraic number of times  $S$  is traversed with  $U_i$  to the left is  $\mu_i - \mu_j$ . But this is also the number of times  $S$  occurs in  $\sum \mu_i \lambda(P_i)$ . Segments bounding one domain only are easily seen to be traversed 0 times, all in all.

If  $C$  is a rectifiable curve with a finite number of complementary domains admitting inscribed polygons, arbitrarily dense in  $C$ , which together with their complementary domains of multiplicities  $\neq 0$  lie in  $D$ , then

$$\iint_C \mu(x, y) u_x(x, y) dx dy = \int_C u(x, y) dx.$$

Since for an inscribed polygon  $P$  the complementary domains approach those of  $C$  as  $P$  gets indefinitely dense in  $C$ , and  $\lim \mu_P(\rho) = \mu_C(\rho)$  for each point  $\rho$  not on  $C$ , we get the theorem from that about closed polygons by the method used at the end of section 3.

5. *Other Formulae of Vector Analysis.*—The method of proving the formula for a rectangular domain as the limit of an identity, and for more

complicated domains by virtue of the elementary continuity properties of the integrals can be applied to many formulae of vector analysis. In this way E. Hemmingsen<sup>4</sup> proved  $\iint (u_x v_x + uv_{xx}) dx dy = \int uv_x dy$ . The Weierstrass sums of the left side contain second difference quotients. The identity underlying the rectangular case can be proved by an Abel transformation of the sum.

<sup>1</sup> Cf. de la Vallée Poussin, *Cours d'Analyse*, pp. 333–342.

<sup>2</sup> Bolza, *Lectures on the Calculus of Variations*, §31. A survey of the results obtained by considering single integrals as limits of their Weierstrass sums is contained in my paper, "Metric Geometry and Analysis," *Rice Pamphlets*, January, 1940.

<sup>3</sup> Cf. H. E. Bray, *Ann. Math.*, **26**, 278 (1925). We do not have to leave the domain bounded by  $C$  if we use (1) the geometric fact that there exists a simple closed polygon in the interior of  $C$  as close as we please to  $C$ , and such that the area of  $C - P$  is arbitrarily small; (2) the continuity of  $\lambda(C)$ , for which an elementary proof can be derived from the author's more general theory (*Ergebn. e. math. Koll.*, **8**, 14–19 (1937)).

<sup>4</sup> In his Master's thesis, University of Notre Dame, 1940.

## SURFACES OF MINIMUM CAPACITY

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1. *Capacities of Surface Caps.*—We adopt the notation of an earlier note, in what follows.<sup>1</sup> In that note it was seen that, given a closed curve  $s$  in space, there exists a unique surface  $S$  bounded by it and, except in the neighborhood of  $s$ , composed of a finite number of smooth regular pieces, which satisfies the analog of the Euler condition for minimum capacity. Except for possible nodal lines and points this surface is analytic. It is assumed that  $s$  is of zero capacity and such that there exists a continuous one-one transformation  $\xi$  of a large sphere containing  $s$  into itself in such a way that each point of the surface corresponds to itself and the points of  $s$  correspond to the points of a circle  $\sigma$  interior to the sphere; for points outside the sphere  $\xi$  may be extended as the identical transformation.

**THEOREM.** *Of all the surfaces which may be considered as caps for  $s$ , the surface  $S$  has the minimum capacity.*

A surface  $S'$  is considered as a cap of  $s$  if (1) every point of  $s$  is a limit point of points of  $S'$ ,  $S'$  is bounded and  $S'$  is composed except in the neighborhood of  $s$  of a finite number of smooth regular pieces,<sup>2</sup> and (2) every closed curve which loops  $s$  contains a point of  $S'$ . We may assume that both sides of every regular surface element of  $S'$  are accessible from  $\infty$ , since otherwise we could diminish the capacity of  $S'$  by removing from

it a finite number of regular pieces of the element, and still retain the property (2).

With unit masses distributed on  $S$  and  $S'$ , respectively, as conductor distributions (that is, with minimum energies) it has to be shown that the energy for  $S$  is greater than that for  $S'$ . These energies may be compared through the Dirichlet integrals. Let us suppose first that the surfaces are sufficiently smooth so that we may apply Green's theorem to the infinite regions  $\Omega$ ,  $\Omega'$  complementary to them, and  $s$  sufficiently smooth so that every point of it is regular with respect to  $\Omega$  and  $\Omega'$ .

Denote by  $\bar{V}(M)$ ,  $\bar{U}(M)$  the conductor potentials of the conductor distributions of unit masses on  $S$ ,  $S'$ , respectively, and by  $\bar{v}(M)$  the harmonic extension of  $\bar{V}(M)$ , according to the method of the cited paper,<sup>1</sup> as a function which is single valued on the two-leaved Riemann space  $\mathfrak{M}$ , harmonic except at points of  $s$ . The function  $\bar{v}(M)$  is two valued at  $\infty$ , one branch taking on the value 0, the other the value  $2/K(S)$  where  $K(S)$  is the capacity of  $S$ . We have

$$D(\bar{V}) = \frac{4\pi}{K(S)}, \quad D(\bar{U}) = \frac{4\pi}{K(S')},$$

where  $D(\bar{V})$ ,  $D(\bar{U})$  are the corresponding Dirichlet integrals. For points on  $S$  we have  $\bar{V} = \bar{v} = 1/K(S)$ , and for points on  $S'$ ,  $\bar{U} = 1/K(S')$ .

2. *Application of Green's Theorem.*—The surface  $S'$  forms a cut surface for  $\mathfrak{M}$  and  $\bar{v}(M)$ , since every closed curve which loops  $s$  contains a point of  $S'$ . Since  $S'$  is bounded, there is a single-valued branch of  $\bar{v}$ , which we denote by  $W$ , harmonic in  $\Omega'$ , and zero at  $\infty$ . We have

$$D(\bar{V}) = \int_{\Omega} (\nabla \bar{V})^2 dM = \frac{1}{2} \int_{\mathfrak{M}} (\nabla \bar{v})^2 dM = \int_{\Omega'} (\nabla W)^2 dM = D(W),$$

for the sum of the two values of  $v(M)$  has everywhere the value  $2/K(S)$ .

Consider now  $D(\bar{U}, W)$ , the mixed integral  $\int_{\Omega'} \nabla \bar{U} \cdot \nabla W dM$ . By an application of Green's theorem we have

$$\int_{\Omega'} \nabla \bar{U} \cdot \nabla W dM = - \int_{S'} \bar{U} \frac{dW}{dn} dP,$$

where the integral of the second member is extended over the complete boundary of  $\Omega'$ , that is, so as to cover the two-sided local neighborhood of every point  $P$  of  $S'$ . But  $\bar{U}$  is constant on  $S'$ , and

$$- \int_{S'} \frac{dW}{dn} dP = - \int_C \frac{dW}{dn} dP = - \int_C \frac{d\bar{V}}{dn} dP = 4\pi,$$



where  $C$  is a sphere which includes the whole of  $S'$  in its interior. Consequently  $D(\bar{U}, W) = 4\pi/K(S')$  and

$$D(\bar{U}, W - \bar{U}) = D(\bar{U}, W) - D(\bar{U}) = 0.$$

But now, writing  $W = \bar{U} + (W - \bar{U})$ , we have

$$D(\bar{V}) = D(W) = D(\bar{U}) + D(W - \bar{U}) + 2D(\bar{U}, W - \bar{U})$$

and

$$D(\bar{V}) = D(\bar{U}) + D(W - \bar{U}),$$

where  $D(W - \bar{U}) \geq 0$ .

3. *Proof of the Theorem.*—In order to treat the case where there may be points of  $s$  which are irregular boundary points of the domains complementary to  $S$  or  $S'$ , we must consider an approximate situation in which  $s$  is replaced by a torus  $\Sigma_n$  surrounding it. Without restricting the generality assumed for  $s$ , it may be assumed that  $\Sigma_n$  is analytic and cuts  $S'$  in a finite number of regular arcs. As in the cited article, the approximate problem is solved for boundary values 1 on the torus, by means of a two-valued harmonic function  $v_n$ , and a surface  $S_n$  is defined as the locus  $v_n = 1$ . We take  $\Sigma_{n+1}$  inside  $\Sigma_n$  and let the Fréchet distance from  $\Sigma_n$  to  $s$  tend to zero as  $n \rightarrow \infty$ .

The conductor potential of the surface formed by  $\Sigma_n$  and the portion  $S_n'$  of  $S'$  outside  $\Sigma_n$ , corresponding to a conductor distribution of unit mass on  $S_n' + \Sigma_n$  is denoted by  $\bar{U}_n$ , and functions  $\bar{V}_n, \bar{v}_n, W_n$  are set up corresponding to unit mass on  $S_n + \Sigma_n$ ,  $W_n$  being defined on the cut space  $\Omega_n'$  outside  $S_n' + \Sigma_n$ . The function  $W_n$  may be taken as constant within  $\Sigma_n$ .

From the fact that  $v_n$  converges to  $v$ , it follows that the integral of the normal derivative for  $V_n$  (on any large sphere) converges to that for  $V$ , and  $K(S_n + \Sigma_n)$  converges to  $K(S)$ , as  $n \rightarrow \infty$ . Hence

$$\lim_{n \rightarrow \infty} D(W_n) = D(W).$$

But also the sets  $S_n' + \Sigma_n$  form a "decreasing" sequence with  $S' + s$  as a limit, and

$$\lim_{n \rightarrow \infty} D(\bar{U}_n) = D(\bar{U}).$$

By writing

$$D(\bar{U}_n, W_n) - D(\bar{U}, W) = D(W_n - W, \bar{U}_n) + D(W, \bar{U}_n - \bar{U})$$



and

$$\frac{1}{4\pi} D(W_n - W, \bar{U}_n) = \int_{S_m'} (W_n - W) d\mu_n + \int_{\Sigma_{m,n}} (W_n - W) d\mu_n$$

$$\frac{1}{4\pi} D(W, \bar{U}_n - \bar{U}) = \int_{S_m'} W d\mu_n - \int_{S_m'} W d\mu + \int_{\Sigma_{m,n}} W d[\mu_n - \mu],$$

where  $\mu_n, \mu$  are the respective distributions of unit mass on  $S_n' + \Sigma_n, S' + s$ , corresponding to the potentials  $\bar{U}_n, \bar{U}$ , and where  $\Sigma_{m,n}$  denotes the portion of  $S_n' + \Sigma_n$  inside  $\Sigma_m$ , we can make use of the weak convergence of  $\mu_n$  to  $\mu$  and the fact that  $s$  is of zero capacity to prove that, by fixing  $m$  great enough and then letting  $n$  become infinite, the two quantities  $D(W_n - W, \bar{U}_n)$  and  $D(W, \bar{U}_n - \bar{U})$  tend to zero. Consequently,

$$\lim_{n \rightarrow \infty} D(\bar{U}_n, W_n) = D(\bar{U}, W), \quad \lim_{n \rightarrow \infty} D(W_n - \bar{U}_n) = D(W - \bar{U}).$$

Thence follows the relation

$$D(\bar{V}) = D(W) = D(\bar{U}) + D(W - \bar{U}).$$

If  $D(W - \bar{U}) = 0$ , it follows that  $W \equiv \bar{U}$  except on  $S' + s$ , and that  $D(W) = D(\bar{U})$ ; that is,  $K(S) = K(S')$ . Let  $P$  be a point not on  $S' + s$ . Then  $W = \bar{U} < 1/K(S)$  and  $P$  is not on  $S$ ; that is,  $S$  lies on  $S' + s$ . Let  $P'$  be a point not on  $S + s$ . Then at  $P'$ ,  $\limsup W \neq 1/K(S)$  and  $\limsup \bar{U} \neq 1/K(S')$ ; and  $P'$  is not on  $S'$ ; that is,  $S'$  lies on  $S + s$ . Hence  $S$  and  $S'$  are identical.

Consequently,  $D(\bar{V}) > D(\bar{U})$  and  $K(S) > K(S')$  unless  $S$  and  $S'$  are identical.

In conclusion, I wish to express appreciation of the considerable assistance which has come through discussion of the problem with my colleague, Professor H. Lewy.

<sup>1</sup> G. C. Evans, "Surfaces of Minimal Capacity," these PROCEEDINGS, 26, 489-491 (1940).

<sup>2</sup> "Smooth," for instance as in Kellogg, *Foundations of Potential Theory*, p. 157, Berlin (1929).

# BOUNDARY VALUES OF FUNCTIONS SATISFYING A LINEAR PARTIAL DIFFERENTIAL EQUATION OF ELLIPTIC TYPE

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1. In the last few years some results and methods of the theory of analytic functions of one complex variable have been taken over for the study of functions  $U(z, \bar{z})$ ,<sup>1</sup> satisfying a linear partial differential equation

$$\begin{aligned} \mathbf{L}(U) &\equiv U_{z\bar{z}} + 2\operatorname{Re}[A(z, \bar{z})U_z] + C(z, \bar{z})U = 0, \\ U_z &= 1/2(\partial U/\partial x - i\partial U/\partial y), \quad U_{\bar{z}} = 1/2(\partial U/\partial x + i\partial U/\partial y) \end{aligned} \quad (1.1)$$

where  $A$  is a complex and  $C$  is a real analytic function of two variables  $z, \bar{z}$  regular in sufficiently large domain  $|z| < \text{const.}, |\bar{z}| < \text{const.}$ <sup>2</sup>

These investigations are based on the following

**THEOREM 1.** *For every  $\mathbf{L}$  there exists a function  $E(z, \bar{z}; t) = 1 + z\bar{z}t^2E^*(z, \bar{z}; t)$  such that every solution,  $U$ , of  $\mathbf{L}(U) = 0$  regular in a star domain  $\mathfrak{F}^2$  (of the  $xy$ -plane) can be represented in  $\mathfrak{F}^2$  in the form*

$$U(z, \bar{z}) = \operatorname{Re} \left[ \int_{-1}^1 E(z, \bar{z}; t) f[1/2 z(1 - t^2)] (1 - t^2)^{-1/2} dt \right] \quad (1.2)$$

where 
$$f(\zeta) = \frac{2}{\pi} \int_0^{\pi/2} \zeta \sin \vartheta \frac{dU(2\zeta \sin^2 \vartheta, 0)}{d(\zeta \sin^2 \vartheta)} d\vartheta \quad (1.3)$$

(the associate of  $U$ ) is an analytic function of one complex variable regular in  $\mathfrak{F}^2$ .

Conversely, the right-hand member of (1.2) with  $f(\zeta)$  regular in  $\mathfrak{F}^2$  always represents a particular solution of  $\mathbf{L}(U) = 0$  regular in  $\mathfrak{F}^2$ .<sup>3</sup>

2. We will now prove the following result, which is of the Fatou<sup>4</sup> type.

**THEOREM 2.** *Let  $f(\zeta) = \sum_{n=0}^{\infty} a_n \zeta^n$  and let*

$$\sum_{n=1}^{\infty} |a_n|^{2n^{-1}} < \infty. \quad (2.1)$$

*Then the function  $U$  given by (1.2) has boundary values almost everywhere on the circle  $|z| = 2$  for radial approach.*

*Proof.*—1°. By (2.1) and by Fatou's theorem for analytic functions of one complex variable, the function

$$g(z) = \int_{-1}^1 f[1/2 z(1 - t^2)] (1 - t^2)^{-1/2} dt = \sum_{n=0}^{\infty} z^n a_n 2^{-n} \int_{-1}^1 (1 - t^2)^{n-1/2} dt = \sum_{n=0}^{\infty} b_n z^n \quad (2.2)$$

has boundary values almost everywhere on the circle  $|z| = 2$  for radial approach since<sup>6</sup>

$$|2^n b_n| \leq |a_n| \int_{-1}^1 (1 - t^2)^{n-1/2} dt = |a_n| \Gamma(n + 1/2) \Gamma(1/2) / \Gamma(n + 1) \leq c_1 |a_n| / \sqrt{n}.$$

2°. Suppose that  $z = 2$  is a convergence point of (2.2). Then (as we will prove below) for every  $\eta > 0$  and for every  $\epsilon > 0$  there exists a  $\delta(\eta, \epsilon)$ ,  $\lim_{\epsilon \rightarrow 0} \delta(\eta, \epsilon) = 0$  ( $\eta$  fixed), so that

$$\left| \int_{-\eta}^{\eta} \left[ \sum_{k=1}^2 (-1)^k f[1/2 r_k (1 - t^2)] \right] (1 - t^2)^{-1/2} dt \right| \leq \epsilon \quad (2.3)$$

for  $|r_1 - r_2| \leq \delta$ ,  $0 \leq r_k < 2$ .

Since  $g(r)$  is continuous in the closed interval  $0 \leq r \leq 2$  (and therefore uniformly continuous) and since  $f(\zeta)$  is regular for  $|\zeta| \leq (1 - \eta^2)$ ,  $\eta > 0$ ,  $\eta$  fixed, our assertion follows from

$$\int_{-\eta}^{\eta} \left[ \sum_{k=1}^2 (-1)^k f[1/2 r_k (1 - t^2)] \right] (1 - t^2)^{-1/2} dt = [g(r_2) - g(r_1)] - \left[ \int_{-1}^{-\eta} + \int_{\eta}^1 \right] \left\{ \left[ \sum_{k=1}^2 (-1)^k f[1/2 r_k (1 - t^2)] \right] (1 - t^2)^{-1/2} dt \right\} \quad (2.4)$$

3°. We proceed to the proof of the existence of  $\lim_{r \rightarrow 2} U(r, r)$ . By (1.2)

$$\begin{aligned} \text{we have} \quad & \left| \sum_{k=1}^2 (-1)^k U(r_k, r_k) \right| \leq \left| \left[ \int_{-1}^{-\eta} + \int_{\eta}^1 \right] \times \right. \\ & \left\{ \left[ \sum_{k=1}^2 (-1)^k E(r_k, r_k; t) f[1/2 r_k (1 - t^2)] \right] (1 - t^2)^{-1/2} dt \right\} \Big| + \\ & + \left| \int_{-\eta}^{\eta} \left[ \sum_{k=1}^2 (-1)^k f[1/2 r_k (1 - t^2)] \right] (1 - t^2)^{-1/2} dt \right| + \\ & + c_2 \sum_{k=1}^2 \left| \int_{-\eta}^{\eta} t^2 f[1/2 r_k (1 - t^2)] (1 - t^2)^{-1/2} dt \right| = \\ & = |I_1(r_1, r_2)| + |I_2(r_1, r_2)| + |I_3(r_1)| + |I_4(r_2)|, \quad c_2 = 4 \max_{\substack{0 \leq r \leq 2 \\ 0 \leq t \leq 1}} |E^*(r, r; t)|. \end{aligned} \quad (2.5)$$

Let  $\epsilon$  be an arbitrarily small positive number. We shall show that we can choose a positive  $\eta$  so small that each  $|I_{2+k}(r_k)|$ ,  $k = 1, 2$  becomes less than  $\epsilon/4$ .

Since  $|f(\zeta)| \leq \left[ \sum_{n=1}^{\infty} |a_n|^2 n^{-1} \cdot \sum_{n=1}^{\infty} n |\zeta|^{2n} \right]^{1/2} \leq c_3 (1 - |\zeta|^2)^{-1}$  we have

$$|f[1/2 r_k(1 - t^2)]| \leq c_3 \left[ 1 - [1/2 r_k(1 - t^2)]^2 \right]^{-1} \leq c_4 t^{-2}, r_k < 2, \text{ and therefore}$$

$$|I_{2+k}(r_k)| \leq 2c_2 c_4 \eta.$$

After choosing  $\eta < \epsilon/8c_2 c_4$  we show in a way analogous to that of 2° that  $|I_1(r_1, r_2)|$  can be made  $< \epsilon/4$ . Furthermore it follows by 2° that  $|I_2(r_1, r_2)|$  can be made less than  $\epsilon/4$ . (In both cases we suppose that  $|r_1 - r_2|$  is sufficiently small.)

Since by 1° the convergence points of  $g(z)$  lie almost everywhere on  $|z| = 2$ , our theorem is proved.

3. Theorem 2 gives a sufficient condition for the existence of radial boundary values almost everywhere in terms of a property of the associate  $f(\zeta)$  of  $U(z, \bar{z}) = U(x + iy, x - iy) = W(x, y)$ .

There arises the question of finding a method which permits us to decide whether (2.1) is fulfilled for  $f$ ,  $W(x, y)$  being given for *real* values of  $x, y$  only. In order to use (1.3) directly one must know the values of  $U(z, \bar{z})$  also if  $z$  and  $\bar{z}$  are not conjugate, i.e., of  $W(x, y)$  if  $x$  and  $y$  are complex.

But by a slight modification of the above considerations we get a criterion for sufficiency. For every  $L$  there exist solutions  $V(\zeta, \bar{\zeta}; z, \bar{z}) = V_1(\zeta, \bar{\zeta}; z, \bar{z}) \log |z - \zeta| + V_2(\zeta, \bar{\zeta}; z, \bar{z})$  of the adjoint equation  $M(V) = 0$ ,  $\zeta \neq z$ ;  $V_1$  and  $V_2$  being regular functions of  $\zeta, \bar{\zeta}$  in a sufficiently large domain.<sup>6</sup> Using the known formula<sup>7</sup> we get the representation

$$U(z, \bar{z}) = 1/2 \pi^{-1} \int_0^{2\pi} [\tilde{U}(\rho, \varphi) K_1(\rho, \varphi; z, \bar{z}) + \tilde{U}_\rho(\rho, \varphi) K_2(\rho, \varphi; z, \bar{z})] d\varphi,$$

$$|z| < \rho, |\bar{z}| < \rho < 2, \tilde{U}(\rho, \varphi) = U(\rho e^{i\varphi}, \rho e^{-i\varphi}), \tilde{U}_\rho(\rho, \varphi) = \partial \tilde{U}(\rho, \varphi) / \partial \rho, \quad (3.1)$$

which is valid also if  $z$  and  $\bar{z}$  are not conjugate;  $K_1, K_2$  being certain functions connected in a simple manner with  $V$ .

By (1.3) and (3.1) we get

$$a_n = 1/2 \pi^{-1} \int_0^{2\pi} [\tilde{U}(\rho, \varphi) K_1^{(n)}(\rho, \varphi) + \tilde{U}_\rho(\rho, \varphi) K_2^{(n)}(\rho, \varphi)] d\varphi,$$

$$n > 0 \quad (3.2)$$

where  $K_p^{(n)}$  are given by

$$\sum_{n=0}^{\infty} K_p^{(n)}(\rho, \varphi) \zeta^n = \frac{2}{\pi} \int_0^{\pi/2} \zeta \sin \vartheta \frac{dK_p(\rho, \varphi; 2\zeta \sin^2 \vartheta, 0)}{d(\zeta \sin^2 \vartheta)} d\vartheta, p = 1, 2.$$

$$(3.3)$$

4. We can obviously use instead of  $V$  Green's function of  $L$  for the circle of radius  $\rho$ . Considerations like those of section 3 yield an analogous

expression for  $a_n$ , where  $\tilde{U}(\rho, \varphi)$  alone appears. For applications, (3.2) is preferable since the properties of Green's function mentioned above are not very well known, while the behavior of the functions  $K_p$  used are comparatively simple.

However in some special cases it is possible to obtain for  $a_n$  expressions where only  $\tilde{U}(\rho, \varphi)$  appears, without using the Green's function of  $L$ .

We suppose now that  $A = 0$  and  $C$  is a function which depends on  $r$ , but not on  $\varphi$ . One sees easily that  $E(z, \bar{z}; t)$  also depends only on  $r$ , and therefore we have for  $U(z, \bar{z})$  the representation

$$U(r, \varphi) = \operatorname{Re} \left[ \sum_{n=0}^{\infty} a_n e^{in\varphi} r^n \int_{-1}^1 \tilde{E}(r; t) \frac{(1-t^2)^{n-1/2}}{2^n} dt \right],$$

$$\tilde{E}(r; t) = E(re^{i\varphi}, re^{-i\varphi}; t) \quad (4.1)$$

We get therefore

$$a_n = \frac{\int_0^{2\pi} \tilde{U}(\rho, \varphi) e^{-in\varphi} d\varphi}{C_n(\rho)}, \quad C_n(\rho) = \rho^n \int_{-1}^1 \tilde{E}(\rho; t) \frac{(1-t^2)^{n-1/2}}{2^n} dt. \quad (4.2)$$

We remark that it can be easily shown<sup>5</sup> that

$$C_n(\rho) \geq c_5 \rho^n 2^{-n} n^{-1/2}, \quad c_5 > 0 \quad (4.3)$$

for  $n$  sufficiently large. We notice furthermore that our procedure leads to various other theorems of Fatou's type for functions  $U(z, \bar{z})$ .

<sup>1</sup> We denote analytic functions of one complex variable  $z$  or  $\zeta$  by small letters, analytic functions of two variables  $z, \bar{z}$  or  $\zeta, \bar{\zeta}$ , respectively, by capital letters;  $z = x + iy$ ,  $\bar{z} = x - iy$ ,  $\zeta = \xi + i\eta$ ,  $\bar{\zeta} = \xi - i\eta$ ;  $x, y$  and  $\xi, \eta$ , respectively, being Cartesian coördinates.

<sup>2</sup> See the following papers: [1] Bergman, Stefan, *Recueil mathématique*, 2 (44) (new series), 1169-1198 (1937); [2] Bergman, Stefan, *Duke Math. Jour.*, 6, 537-561 (1940).

We suppose that  $A$  and  $C$  can be analytically prolonged in a certain domain of the complex variables  $x$  and  $y$ . (Notice that for  $x$  and  $y$  complex,  $z$  and  $\bar{z}$  are not longer conjugate.)

<sup>3</sup> See Bergman, *Recueil mathématique*, 2 (44), (new series) 1172-1179 (1937).

<sup>4</sup> We notice that criteria for the existence of boundary values almost everywhere are obtained in some cases immediately from an analogous theorem for subharmonic functions. (Cf. Privaloff, I. I., and Kouznetzoff, P., *Recueil mathématique*, 6 (48) (new series), 345-376 (1939).) If we suppose that  $U$  is non-negative in the unit circle and  $A = 0$ , then we can divide the circle into a finite number of regions so that in every region  $U$  is either super- or subharmonic and we then use the theorem cited.

<sup>5</sup> See Nielsen, N., *Handbuch der Theorie der Gammafunktionen*, Leipzig, 1906, p. 92. We denote by  $c_5$  appropriate constants.

<sup>6</sup> See Hedrick, R., *Ueber den analytischen Charakter der Lösungen von Differentialgleichungen*, Dissertation, Göttingen, 1901.

<sup>7</sup> See Sommerfeld, A., *Enzykl. der math. Wissensch.*, II A, 7c, 504-570 (1900-1916), especially p. 515.

<sup>8</sup> See Bergman, *Recueil mathématique*, 2 (44) (new series), 1181 (1937).





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*THE DEVELOPMENT OF MELANOPHORES FROM EMBRYONIC  
MOUSE TISSUES GROWN IN THE COELOM OF CHICK EMBRYOS*

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Transplantation experiments have demonstrated conclusively that migratory pigment-forming cells originate from the neural crest in amphibians<sup>2</sup> and in birds;<sup>3,4,11</sup> but so far no attempt has been made to determine whether or not the same is true in mammals. In their studies on the origin of pigment in birds Eastlick<sup>4</sup> and Ris<sup>11</sup> found the method of grafting embryonic tissue to the coelomic cavity of a developing chick embryo as used by Hamburger<sup>5</sup> particularly suitable. As far as the author is aware, tissues other than those of birds have not been grown in the coelom, but it is known that embryonic tissue of both the rat<sup>7,9</sup> and the mouse<sup>10</sup> will differentiate to a certain extent on the chorio-allantoic membrane of the chick. The possibility that the intracoelomic grafting method might be used with success for testing the origin of pigment cells in a mammal seemed too tempting to leave untried, therefore the following experiments were undertaken.

*Experimental Procedure.*—The mice from which the donor embryos were taken were of a strain known to produce only black offspring.<sup>14</sup> Embryos were used at ages ranging from 8½ to 12 days, timed from the observance of the vaginal plug. They were removed from the uterus (under anesthesia) one at a time as needed. Those not used were fixed as controls for histological study. Each donor embryo was placed in warm sterile Locke's solution in a small Petri dish placed under a binocular dissecting microscope. After all membranes had been removed and the somites counted, small pieces (0.5 to 1 mm. in length) of skin ectoderm, plus some of the mesoderm lying directly beneath it, were isolated from various levels along the antero-posterior axis and implanted one at a time into the coelomic cavity of White Leghorn host embryos of 60 to 70 hours' incubation. White Leghorns were chosen as hosts for these experiments because of the fact

that they do not regularly exhibit pigment in their coelomic epithelium. Only 10 individuals out of 115 examined especially for this purpose on the 18th or 19th days of incubation showed even a trace of pigment. When present the pigment was more or less evenly distributed in the coelomic epithelium with greatest concentration in the region of the umbilicus. Examination of the pigmented region shows that nearly all of the melanophores had undergone degeneration, i.e., had retracted their processes, and persisted only as small dense balls of pigment (Fig. 5). For an account of similar degeneration in regenerated feathers and *in vitro* cultures see Hamilton.<sup>6</sup>

When the donors had well-developed limb buds (at 11 and 12 days), skin ectoderm plus adhering mesoderm from these regions as well as that from the head and trunk was tested. In the younger stages, however, the entire limb bud or longitudinal halves of limb buds were grafted, as were also pieces of somite and lateral plate with and without the neural tube. Aside from the intracoelomic implantations, a few grafts were made to the base of the developing wing bud. For this purpose skin ectoderm as free as possible of adhering mesenchyme was taken from the posterior head region of 10-day embryos (about 30 somites).

For making the isolations, steel beading needles with very fine points, sealed into glass tubing for handles, and a Bowman's iris knife were used. To facilitate handling, the pieces were stained by touching them lightly, before removal from the body of the embryo, with small glass rods having agar-coated tips stained with Nile blue sulphate.

The preparation of the host embryo was essentially that described previously for transplanting embryonic chick tissue to the body wall or to the limb bud.<sup>18</sup> A small rectangular piece of shell approximately  $1 \times 0.5$  cm. was sawn and lifted out immediately over the body of the embryo whose position had been previously determined by candling. The shell membrane was picked away carefully under a binocular microscope, thus exposing the embryo lying on the yolk beneath. By touching the embryonic area with the stained tips of the above-mentioned glass rods, the membranes become visible immediately and can be very easily opened. Usually at the stages used the amnion had not grown over the posterior regions, so did not have to be dealt with. When the body wall was thus exposed a small slit was made with a glass needle in the ectoderm and somatic mesoderm just anterior to the leg bud region of either the right or left sides. The implant of donor tissue was transferred through the window to the host blastoderm in a capillary pipette of suitable size with a small amount of saline solution. It was pushed through the slit gently with a glass needle into the coelomic cavity. The original piece of shell was fitted into the window the edges sealed with melted paraffin and the egg returned to the incubator. During the operation and afterwards the egg was placed on a cotton pad in a

Syracuse watchglass to prevent rotation. The host embryo was removed and dissected usually on the 19th day of incubation. A thorough examination of the coelom was made for the graft and melanophores migrating from it. In all positive cases the grafts were fixed *in situ* in Bouin's fluid for histological study.

*Observations.*—When the hosts were dissected, it was found that the implanted mouse tissue had differentiated into a definite body or graft in 62 out of 101 cases. Except when entire limb buds or halves of limb buds had been taken originally, the grafts were small, rounded or oval-shaped bodies not exceeding 4 mm. in diameter, well vascularized and firmly attached to the wall of the coelom in the posterior region of the body. Often the free surface of the graft was connected to mesenteries, intestine, colocal wall or even to some other part of the coelomic epithelium by strands of connective tissue. Always the graft surface was covered with a thin, smooth, transparent layer of epithelium. The great majority of grafts contained well-developed hairs, very conspicuous when pigmented (Fig. 1), less so when unpigmented. Due to the fact that the implanted piece of skin ectoderm always tends to round up into a hollow ball or vesicle, the developing hairs are most often directed inwards towards the center of the graft.

The total age of the graft tissue when removed for study is equivalent to that of 3- to 7-day postnatal mice ( $8\frac{1}{2}$  to 12 days + approximately 16 days in the coelom) and of strictly comparable differentiation. At these ages pigmented hairs can be seen in the normal mouse and with low magnification numerous melanophores scattered in the skin between the hairs are easily visible. Even at birth melanophores concentrated in the developing hair follicles and distributed in the skin between them can be found in the dorsal and lateral regions of the body. In 34 cases specific mouse melanophores densely crowded with coarse black melanin granules were found in the graft or migrating in the coelomic epithelium of the host in its immediate vicinity (Figs. 2, 3 and 4).

The larger size of the mouse melanophore as compared with that of the White Leghorn, and its extreme blackness make it very conspicuous and easy to detect in the coelomic epithelium. Usually there are two diametrically opposed processes ranging from 50 to 100 micra in length (Fig. 3). Occasionally several cells hang together and form long chains (Fig. 4).

Whether or not melanophores were produced by the grafted tissue was soon found to depend upon the age and the region of the embryo from which the implant was taken. Differences in these respects are indicated in the following paragraphs, grafts from older embryos being described first.

*Twelve-Day Donors.*—Skin ectoderm plus adhering mesoderm from all regions tested, namely, neck, shoulder, rump and limb buds, gave 15

successful grafts out of 20 transplants. All had well-developed pigmented hairs, many approximately 1 mm. long (Fig. 1). Histological study of one typical graft showed a vesicular arrangement of well-differentiated skin with numerous hairs growing out into a central cavity. Melanophores were seen in the subcutaneous tissue and dermal layer of the skin as well as in the hair follicles themselves. Although all of the grafts contained pigment, only 9 of the 15 showed migration of melanophores into the surrounding coelomic epithelium of the host and in none was it very extensive.<sup>16</sup>

*Eleven-Day Donors (40–45 Somites).*—Eleven grafts were recovered from 22 hosts examined. As in the preceding series, skin ectoderm and mesoderm from the head and trunk levels produced hairs with pigment, and in 5 of the 7 cases rather extensive migration of mouse melanophores into the host coelomic epithelium was observed. Unlike the preceding, however, skin ectoderm and mesoderm from the limb buds (4 cases, 2 examined histologically) gave well-differentiated hair follicles wholly without pigment: no melanophores were found either in the graft or in the host coelomic epithelium.

*Ten to 10<sup>1</sup>/<sub>2</sub>-Day Donors (28–35 Somites).*—In addition to transplanting

#### DESCRIPTION OF PLATE 1

Figure 1. Graft developed from small piece of skin ectoderm + adhering mesenchyme (0.8 × 0.5 mm.) from trunk at base of right fore limb bud of a 12-day mouse embryo, grown 16<sup>1</sup>/<sub>2</sub> days in the embryonic coelom of a White Leghorn chick. Note mass of well-developed hairs fully pigmented. Photographed *in situ*. (× 10.)

Figure 2. Portion of coelom of White Leghorn host embryo (19<sup>1</sup>/<sub>4</sub> days) showing migration of mouse melanophores from graft (G). Note concentration of melanophores along walls of small blood vessels. Produced by implanting right half of neural tube and adjacent somite material at 25th somite level of 10-day (31 somites) mouse embryo to 70-hr. chick coelom. (× 10.)

Figure 3. Unstained whole mount of portion of coelomic epithelium of White Leghorn host embryo showing migrating mouse melanophores produced from implant of hind limb bud skin ectoderm (0.7 × 0.3 mm.) from 12-day mouse embryo, grown 16<sup>1</sup>/<sub>2</sub> days in embryonic chick coelom. Graft (not shown) contained hairs fully pigmented. (× 85.)

Figure 4. Unstained whole mount of portion of coelomic epithelium of a White Leghorn host embryo showing migrating mouse melanophores produced from dorsal trunk skin ectoderm (0.7 × 0.4 mm.) of 45-somite (11-day) embryo, grown 17<sup>1</sup>/<sub>2</sub> days in coelom. Note melanophores hanging together in long chains. (× 85.)

Figure 5. Unstained whole mount of portion of coelomic epithelium of a normal 19-day White Leghorn embryo showing degenerated melanophores with retracted processes. (× 85.)

Figure 6. Portion of wing of 17-day White Leghorn host embryo showing dense mass of mouse melanophores in the skin and subcutaneous tissue. Produced by implanting head skin ectoderm (1 × 0.4 mm.) from a 10-day (30-somite) mouse embryo to the wing bud region at 60 hrs. of incubation. (× 25.)

Figure 7. Same as figure 6 with greater magnification showing mouse melanophores at the periphery of mass. (× 85.)



PLATE 1





skin ectoderm and subjacent mesoderm from various levels as done in the preceding series, grafts were made also of entire limb buds, longitudinal halves of limb buds, short segments of somite and somite plus the adjacent half of the neural tube. Thirty grafts were obtained from 40 hosts examined; 6 were studied histologically.

Head skin and trunk skin from the dorsal region, i.e., directly over the neural tube, at all levels produced melanophores both in the graft and in the coelomic epithelium about the graft. But no melanophores developed from either of the limb buds when grafted in their entirety or in part, although well-differentiated hair follicles were abundant. The entire limb bud (12 cases) showed a remarkably normal differentiation of skeletal parts, muscle, skin and hair.

The most beautiful and extensive migration of mouse melanophores into the coelomic epithelium came from grafts of somite including the neural tube taken from the leg bud level or just anterior to it. In the best of the six cases obtained, melanophores in large numbers had migrated for a distance of about 5 mm. in two directions along the walls of small blood vessels (Fig. 2). The grafts themselves showed no pigment either macroscopically or microscopically. In the two studied histologically small skin vesicles with well-differentiated skin were found but no hair follicles. The bulk of the graft consisted of a mass of central nervous tissue (spinal cord) showing strikingly normal differentiation.

Similar grafts obtained from the intact somite not including the neural tube showed no pigment either in the graft or in the coelomic epithelium (6 cases). Histological examination of two of these showed good development of skin and hair follicles. One in addition contained skeletal muscle and small cartilages.

In view of the fact that the developing wing bud of the chick has proved such a favorable site for melanophore migration in birds in general, it seemed desirable to make the same test with mouse melanophores. Thus small pieces of head skin ectoderm relatively free of adhering mesoderm were grafted to the wing bud region of White Leghorn host embryos of approximately 60 hours' incubation. In one case out of three, examination on the 17th day showed a small black spot 2 mm. in diameter, in the skin and subcutaneous tissue of the wing of the host at the implantation site (Fig. 6). When examined under a microscope the black area was found to be densely crowded with large branching mouse melanophores exactly like the normal and like those developed in the coelom (Fig. 7). None of the melanophores had migrated into the overlying feathers.

*Eight and One-half to 9-Day Donors (15-20 Somites).*—Embryos at this stage had not developed limb buds and were very fragile. Two types of transplants were made: those in which the somites and lateral plate were cut entirely free of the neural tube, and those in which the neural tube was

left attached. Levels from the 10th somite posteriorly were thus tested. Six grafts were recovered from eight hosts alive at the 19th day of incubation; two came from implants of pure somite and lateral plate and produced no pigment; the other four from implants of somite and lateral plate including neural tube. In none of the four containing neural tube was pigment found in the graft itself, but two showed typical mouse melanophores migrating in the coelomic epithelium around the graft site. In one, the melanophore migration was particularly pretty and extended for several millimeters along a strand of tissue attaching the graft to the cloacal wall. Even though the number of grafts analyzed in this series is small, the results are definitely in line with the others.

*Discussion.*—The results summarized above show that between  $8\frac{1}{2}$  and 12 days of gestation the capacity of the grafted embryonic tissue to produce melanophores spreads rapidly both antero-posteriorly and medio-laterally. Grafts from the head region at all stages tested produced pigmented hairs consistently (no data on head skin of  $8\frac{1}{2}$ -day embryos), but whether or not grafts from the somites and limb buds produced hairs with pigment depended upon both age and body level. For example, at  $8\frac{1}{2}$  days no melanophores developed from grafts of somite material when completely isolated from the neural tube; at 10 days skin ectoderm and mesoderm from somites of the anterior trunk levels including the fore limb region gave hairs fully pigmented while similar grafts from the hind limb region gave hairs entirely without pigment; at 11 days somite grafts from all levels including the posterior limb region produced hairs with pigment. Ten and 11-day limb buds, entire or in part, failed to develop pigment, although skin and hair follicles were abundant. By 12 days, however, pigmented hairs developed regularly from implants of skin + adhering mesenchyme from both limb buds.

This occurrence of well-differentiated hairs wholly without pigment from certain body regions at certain developmental stages suggests strongly that the melanophores migrate into the developing hair follicles from an outside source. That this source lies in or is closely associated with the neural tube is demonstrated by the fact that at those stages in which somite material failed to produce pigment cells, as in the hind limb region of a 10-day embryo, for example, the same regions would develop pigment if the implant was made to include the adjacent part of the neural tube.

The results of the present experiments parallel very closely those obtained from similar transplantation experiments in the chick. By grafting limb buds from chick embryos (24–30 somites) of pigmented breeds to the coelom of White Leghorn hosts of similar ages, Eastlick<sup>4</sup> was able to show that white feathers only were produced unless the implant included body wall material (skin ectoderm and mesoderm) up to the neural tube. Ris<sup>11</sup> obtained similar results by transplanting to the chorio-allantois as well as

to the coelom. Limb buds from embryos of pigmented breeds isolated at 72 hrs. developed white feathers at both sites while the same isolations later, i.e., at 90 hrs. or more, produced pigmented feathers in the graft.

Further evidence for the extra-epidermal origin of pigment cells in the chick, and their lateral migration from the neural tube region into the overlying ectoderm and into the limb buds, is obtained from the transplantation experiments of Willier and Rawles,<sup>18</sup> and Watterson.<sup>12</sup> Small pieces of skin ectoderm or pure mesoderm from embryos of one breed were implanted into the developing wing bud region of host embryos of a similar age (67–108 hrs.) but of a genetically different breed. Implants from the head or trunk regions at all ages tested produced an area of donor-colored feathers on the host at and about the site of implantation. But implants from the wing bud of embryos younger than 80 hours or from the leg bud earlier than 96 hours failed to produce pigment in the host feathers.

Using the same method of implantation to the limb bud, Dorris<sup>2</sup> obtained areas of pigmented skin and feathers in white hosts by implanting thin strips of the rising neural folds (neural crest region) anterior to the first somite from embryos of 3–10 somites of black breeds. Ris<sup>11</sup> correlated the development of pigment in grafts with the morphological appearance of the neural crest at the time of isolation. By transplanting portions of embryos (potentially pigmented) from various levels at successive stages in their development to the embryonic coelom of White Leghorns, he was able to show quite convincingly that the inclusion of the neural crest, migrating cells of the crest or presumptive neural crest, in the transplant was essential for the development of melanophores.

The similarity of the intra-coelomic grafts of mouse tissue with those obtained by Ris from chick and other bird implants is indeed striking. While the mouse melanophores do not migrate nearly as extensively in the White Leghorn coelomic epithelium as those from birds, they do nevertheless tend to wander out from the grafts and follow blood vessels, nerves and strands of connective tissue in much the same way. The failure of mouse melanophores to migrate to any extent in the wing bud, as observed in the one case obtained, is in contrast with the tremendous migration exhibited by bird melanophores in the same site.

The extensive work of Holmdahl<sup>8</sup> on the origin and development of the neural crest in birds and mammals shows how very much alike these two groups are in respect to this structure. In both it arises first in the region of the mid-brain at early somite stages, and develops posteriorly along the neural tube, migrating laterally as the age of the embryo increases. Histological examination of control mouse embryos at the stages and levels used for transplantation shows that the regions which produced pigment in the grafts coincide with the regions from which the neural crest had already migrated from the neural tube. At stages where in posterior levels

the crest had not migrated, or had not migrated sufficiently far to be included in the transplant ( $8\frac{1}{2}$ –10 days), no pigment resulted. Such facts strongly suggest that the same source and mode of migration of melanophores obtains in the mouse as in the bird.

In summary, the experimental as well as the morphological evidence shows that in the mouse, as in the chick, the melanophores arise from an outside source, the neural crest, presumably, and migrate into the skin and developing hair follicles. Further, it is demonstrated that the embryonic coelom of the chick is an excellent site for the growth and differentiation of implanted embryonic mammalian (mouse) tissue.

<sup>1</sup> The experiments upon which this report is based were carried out at the University of Rochester. The manuscript was prepared at Stanford University during a stay of four months in the laboratory of Professor C. H. Danforth. I am deeply indebted to him for the interest he showed in examining the material and for many helpful suggestions. My appreciative thanks are also due Professor B. H. Willier for a critical reading of the manuscript.

<sup>2</sup> Dorris, F., *Jour. Exp. Zool.*, **80**, 315–345 (1939).

<sup>3</sup> DuShane, G. P., *Ibid.*, **78**, 485–501 (1938).

<sup>4</sup> Eastlick, H. L., *Ibid.*, **82**, 131–158 (1939).

<sup>5</sup> Hamburger, V., *Ibid.*, **77**, 379–397 (1938).

<sup>6</sup> Hamilton, H. L., *Anat. Rec.* **78** (1940).

<sup>7</sup> Hiraiwa, Y. K., *Jour. Exp. Zool.*, **49**, 441–457 (1927).

<sup>8</sup> Holmdahl, D. E., *Zeit. mikro-anat. For.*, **14**, 99–298 (1928).

<sup>9</sup> Nicholas, J. S., and Rudnick, D., *Ibid.*, **66**, 193–256 (1933).

<sup>10</sup> Reed, S. C., and Alley, A., *Anat. Rec.*, **73**, 257–265 (1939).

<sup>11</sup> Ris, Hans, *Physiol. Zool.* (in press) (1941).

<sup>12</sup> Watterson, R. L., *Anat. Rec.*, **70**, Supp. 4, 100 (1938).

<sup>13</sup> Willier, B. H., and Rawles, M. E., *Physiol. Zool.*, **13**, 177–199 (1940).

<sup>14</sup> Heterozygous black females were crossed with homozygous black males (C57 black Bar Harbor strain) and the  $F_1$  females from this mating back-crossed to the male parent for successive generations.

<sup>15</sup> A few trial cultures of skin ectoderm were grown *in vitro* in a medium of chick embryonic extract and plasma by Mr. Howard L. Hamilton. Both hair follicles and melanophores differentiated in the explants.

*GALACTIC AND EXTRAGALACTIC STUDIES, XI. NOTE ON  
THE PERIOD FREQUENCY OF GALACTIC CEPHEIDS*

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Communicated November 14, 1940

*1. Introduction.*—The striking correlation of the period lengths of classical Cepheids with the density of the star fields in which they lie was described for the Small Magellanic Cloud in the fifth paper of this series.<sup>1</sup> The large number of variables for which periods had been determined permitted the examination of the frequency of periods separately for various sections of the system. Both the mean period and the median period show the dependence on distance from the nucleus of the Cloud, the longer-period stars being preferentially in the region of high star density and high gravitational potential.\*

The correlation is neither strongly confirmed nor denied by the available material for the periods of Cepheids in the Large Magellanic Cloud; further work is in progress to provide a competent test. The distribution of the periods of Cepheids in our own Galaxy was reexamined some time ago, but little weight could be given the available evidence because of the strong selection factors that operate against a homogeneous collection of material. Irregular and undetermined amounts of space absorption make it impossible to determine the true magnitudes and distances of the faint galactic Cepheids; and, furthermore, the discovery surveys are complete only in limited areas, and there are complications with the boundaries of the Galaxy in the anticenter direction and in high latitudes. These boundary questions arise, of course, from the interdependence of period and intrinsic luminosity.

The problem of the distribution of galactic Cepheids is now taken up again because many new periods have been obtained recently for faint stars in the direction of the galactic center. From this new material strong support is obtained for the hypothesis that the period-field density correlation found in the Small Magellanic Cloud may be a general property of Cepheid variation.

*2. Faint Classical Cepheids near the Galactic Center.*—In various earlier papers Miss Swope and I have reported on the variable stars in certain fields in Sagittarius, Scorpio and Ophiuchus, in the general direction of the central nucleus of the Milky Way.<sup>2</sup> Long-period variables appear in the fields most numerous, but cluster-type Cepheids and eclipsing stars are also abundant, and this region is the "home" of the faint galactic novae. The peculiar distribution of the various types reflects on the absolute magnitudes of the different kinds of variables and also on the distribution of



obscuring matter in this section of the Milky Way. Classical Cepheids are, however, relatively scarce in these great southern star clouds. In fact, the relative frequency of cluster-type and classical Cepheids in the direction of the galactic nucleus is much the same as it is in many of the globular star clusters, where there are scores of cluster-type variables and only two or three Cepheids of long period.

Probably obscuration prevents our low-latitude survey from reaching into the regions of the Milky Way beyond the central nucleus. The variables that are found by the thousands in low latitudes throughout this fourth quadrant of longitude ( $270^\circ$  to  $360^\circ$ ) are either in the rich nuclear star clouds or between them and the Sun.

Variable-star surveys have been carried through with reasonable completeness to magnitude 16.5 (or occasionally fainter with plates made with the Bruce refractor) in the following Milky Way fields, for which the approximate galactic coördinates of the centers are given:

MWF	LONGITUDE,	LATITUDE,
184	315	-7.5
185	322.5	+7.5
186	322.5	0.0
187	322.5	-7.5
189	330	0.0

In table 1 the names, galactic coördinates, median magnitudes and periods are given for the classical Cepheids in these five fields. Of the thirteen stars in MWF 184 only CQ Scorpii has been published, but the others will appear in a forthcoming *Harvard Observatory Bulletin*, with magnitudes, light elements and equatorial coördinates derived by Miss Constance Boyd, who discovered eleven of the thirteen variables. The light curves of all thirty-three variables have been determined from Harvard plates. Four of them, AL CrA, V532 Sgr, V557 Sgr, IU Sco, have unusual curves, and the periods of the second and fourth are the longest in the whole list; but probably all are classical Cepheids.

Since the direction to the galactic center is very near to longitude  $327^\circ$ , latitude  $0^\circ$ , it appears that the five fields here under consideration, which cover a total of about two hundred and eighty square degrees, are all closely involved with the galactic nucleus.† The positions are shown on the accompanying small-scale photograph of the nuclear region (Fig. 1). On this photograph the boundaries of the fields have been roughly sketched in and also lines indicating the galactic coördinates. The small number of classical Cepheids in MWF 186 presumably results from the heavy obscuration along the galactic equator.

MWF 184 is the field farthest from the center. None of its classical Cepheids is in the corner of the field toward the galactic circle, apparently



because heavy intervening obscuration affects that region. The long period variables for this field have been published<sup>3</sup> and show a similar avoidance of the obscured region. In figure 2 the positions of the long-period variables are indicated by dots and the positions of the classical Cepheids by circles.

TABLE 1  
THIRTY-THREE CLASSICAL CEPHEIDS IN MWF 184, 185, 186, 187, 189

NAME	FIELD	$\lambda$	$\beta$	$m$	PERIOD $d$
X Sgr	189	329°	— 1°	5.35	7.012
W Sgr	189	329	— 5	5.4	7.59
BF Oph	185	325	+ 8	8.2	4.068
HV 7902	186	322	— 1	8.5	4.528
RY Sco	187	324	— 5	8.95	20.31
HV 10513	184	314	— 11	12.2	19.818
V626 Sgr	187	325	— 8	12.35	26.762
AL CrA	187	323	— 11	12.5	17.061
AV Sgr	189	335	— 2	12.6	15.411
ET Oph	185	324	+ 8	12.8	25.22:
HV 7885	186	317	— 1	12.95	16.263
V564 Sgr	187	325	— 8	13.1	27.8
CQ Sco	184	319	— 7	13.55	30.412
HV 10260	189	333	+ 3	13.55	14.865
HV 10460	184	315	— 5	13.7	11.155
CE Oph	185	324	+ 10	13.95	15.89
V446 Sco	187	322	+ 3	14.0	28.634
HV 10302	189	331	— 2	14.15	5.748
V532 Sgr	187	323	— 7	14.35	34.21
HV 10495	184	317	— 8	14.4	15.234
HV 10469	184	313	— 8	14.4	1.176
HV 10488	184	315	— 8	14.45	24.693
HV 10456	184	312	— 6	14.5	11.821
HV 10505	184	319	— 8	14.75	16.547
HV 10246	189	331	+ 3	14.8	29.533
V557 Sgr	187	323	— 8	14.8	16.260
HV 10512	184	318	— 9	15.1	15.632
HV 10467	184	317	— 5	15.2	15.66
HQ CrA	187	322	— 11	15.4	1.415
HV 10485	184	318	— 7	15.5	15.526
IU Sco	185	320	+ 6	15.55	69.05
HV 10484	184	319	— 7	15.55	24.09
HV 10509	184	317	— 9	16.0	2.441

In none of the five fields have we found external galaxies, bright or faint.<sup>4</sup> We must assume, therefore, that the total photographic absorption may be several magnitudes in the most obscured regions and perhaps two magnitudes in the richest star fields that are without visible dark lanes.

3. *The Abnormal Frequency of Periods.*—From table 1 it is seen that the

frequency of periods is unlike that found elsewhere, even in the nucleus of the Small Magellanic Cloud. The mean period for the thirty-three stars is 17.94 days, and the median value is 15.99 days. Dropping the extreme value 69.05 days for IU Scorpil, we have the mean and median periods 16.34 and 15.78 days, respectively. Figure 3 illustrates this unusual preference for long periods, with the remarkable clustering at sixteen days. The median apparent brightness for the thirty-three stars is 14.0; for the eleven with periods between 14.8 and 17.1 days it is 14.4.

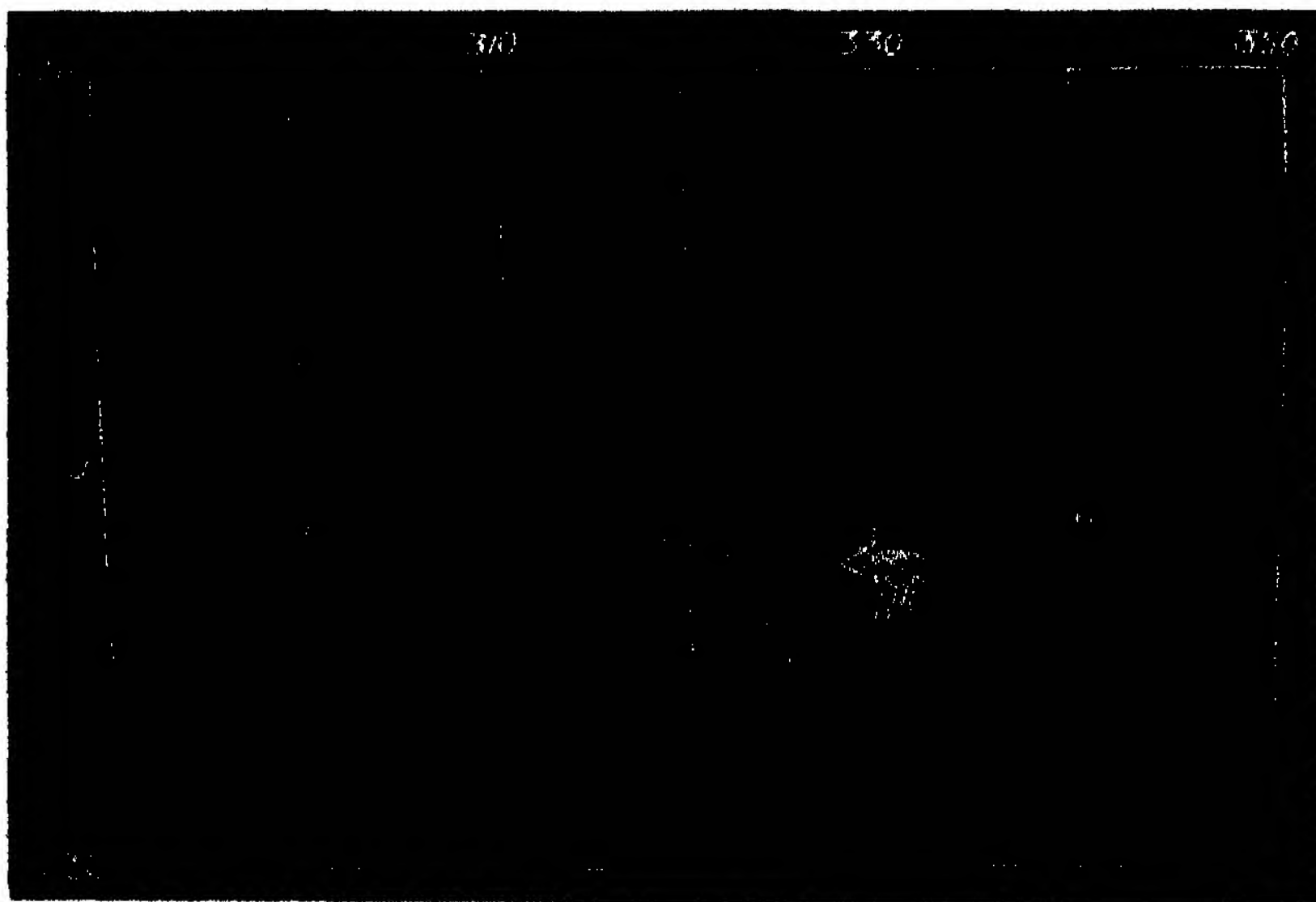


FIGURE 1

Composite photograph of the galactic nucleus showing location of Milky Way fields. Only one-third of MWF 185 has been thoroughly studied. The ordinates are galactic latitudes; the abscissae, galactic longitudes. The center is at  $0^\circ, 327^\circ$ , approximately.

The question arises at once whether or not some factor of selection is responsible in this survey for the infrequency of Cepheids with periods from three to seven days—the interval that includes 51 per cent of the 340 classical galactic Cepheids now known and catalogued. Many cluster-type and eclipsing variable stars have been found that are more than a magnitude fainter than the Cepheids with sixteen-day periods; and since Cepheids of five-day period are intrinsically less than a magnitude fainter, these ordinary Cepheids should have readily been discovered if present. It is necessary to conclude, therefore, that the scarcity is real and that very few Cepheids of ordinary period exist in the fields around the galactic center.

The situation in MWF 184 is specially worth noting. The median mag-

nitude of the thirteen classical Cepheids is 14.5, and the median period is 15.6 days. Only two periods are shorter than eleven days, whereas, in the same field, intermingled with these classical Cepheids, are many cluster-type Cepheids with median magnitudes from 15.0 to 16.1. Since the classical Cepheid with a period of a few days is, when present, an easy variable to discover on the Harvard photographs, we must conclude that such stars are essentially absent from MWF 184.

The total material is not large but appears quite sufficient to demonstrate that the classical Cepheids in the vicinity of the galactic center show the same tendency to high luminosity, large mass and long period, as is shown by the classical Cepheids in the center of the Small Magellanic Cloud.

Because of uncertainty as to the total amount of intervening space absorption, we cannot be sure that these long-period Cepheids are actually as distant as the galactic nucleus, that is, nine or ten kiloparsecs distant. But the observed differences between the average magnitudes of the long-period variables, cluster-type Cepheids and classical Cepheids in the same fields are consistent with that assumption.

The distance modulus,  $m' - M$ , corresponding for MWF 184 to the median observed magnitude 14.5 and the median

period 15.6 days, is 16.85. To justify the assumption that these long-period Cepheids are closely associated with the galactic nucleus, and therefore at a distance of approximately ten kiloparsecs, we would require for this "median Cepheid" a space absorption of  $16.85 - 15.0 = 1.85$  magnitudes. This is perhaps an acceptable value for the bright star clouds, although much larger absorption is probable in the adjacent rifts along the galactic equator.

4. *Galactic Longitude and Period Length.*—In the 1940 catalog of variable stars, issued from the Berlin-Babelsberg Observatory, there are 336 variables listed as classical galactic Cepheids with galactic latitudes lower than  $\pm 30^\circ$  (only three now listed are in higher latitudes, except for a number in the vicinity of the Magellanic Clouds that actually belong to those systems and not to our own Galaxy). The distribution of these 336 vari-

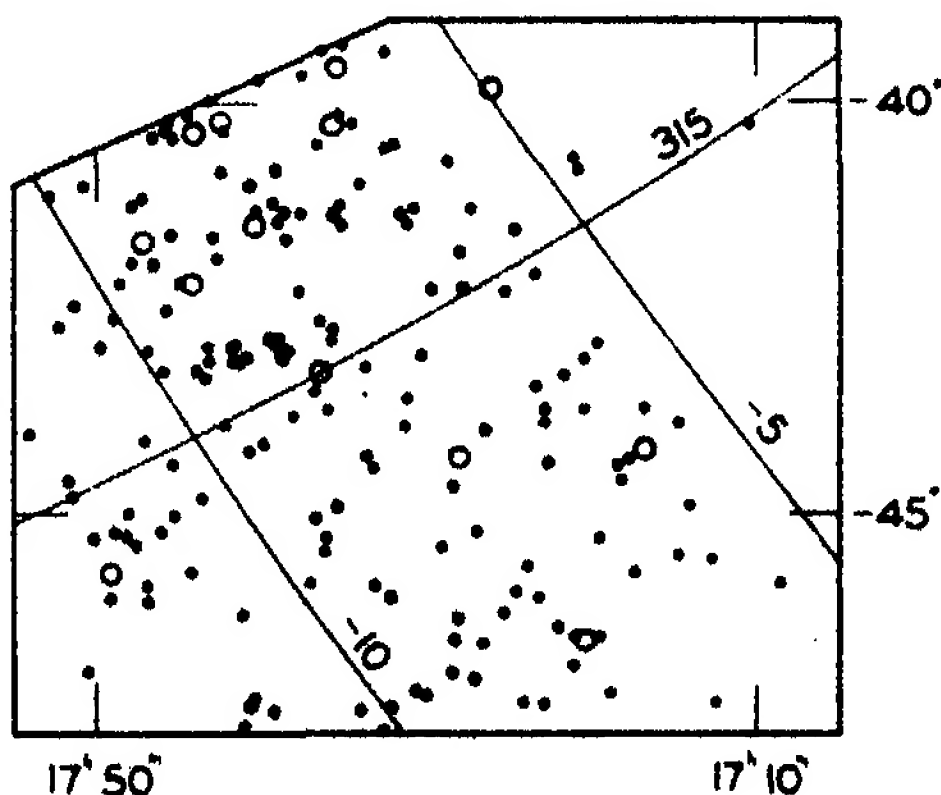


FIGURE 2

Distribution of long-period variables (dots) and classical Cepheids (open circles) in MWF 184. Galactic coördinates are labelled inside the figure; equatorial coördinates on the outside.

ables in thirty-degree intervals of galactic longitude is shown in table 2, which includes also for each group the median period,  $\bar{P}$ , and the mean

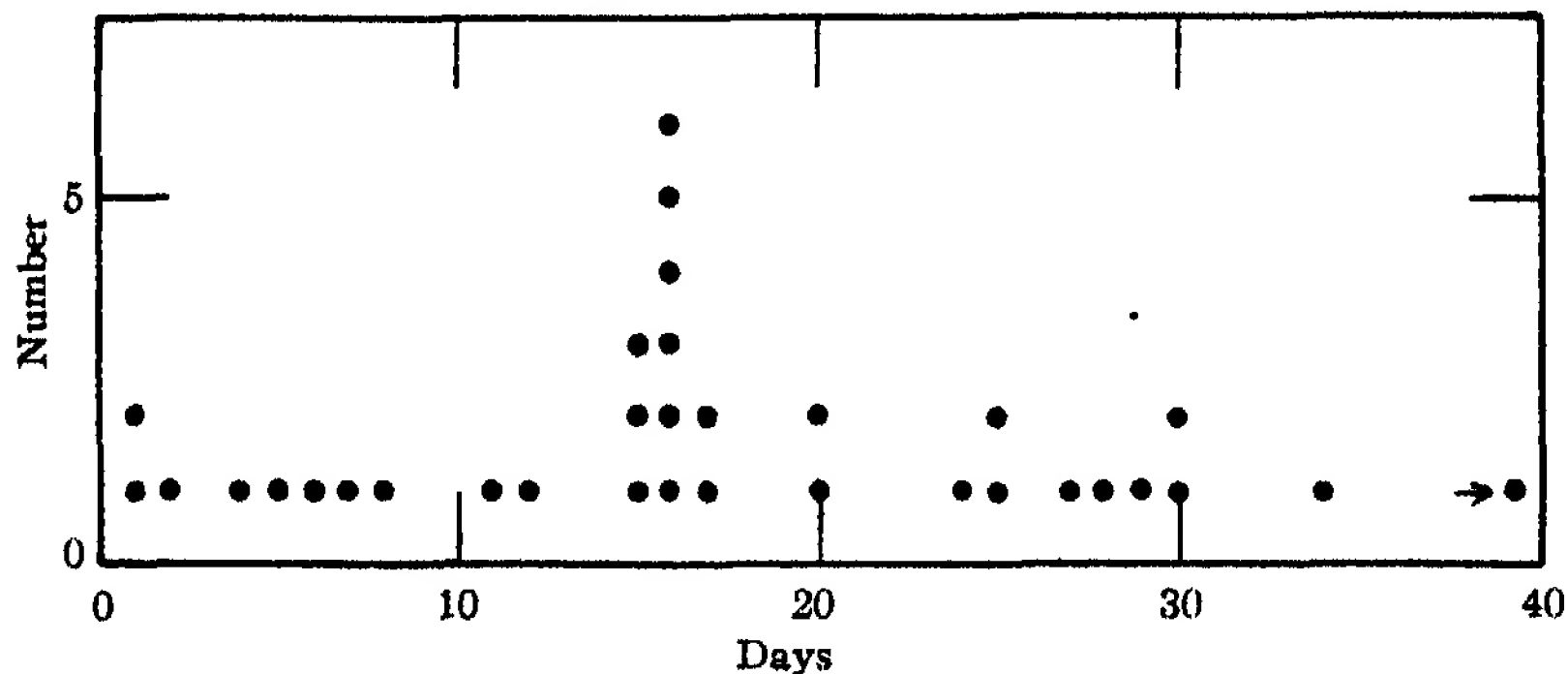


FIGURE 3

Distribution of periods (to nearest whole day) for the thirty-three classical Cepheids in MWF 184, 185, 186, 187, 189. The long period is 69.05 days.

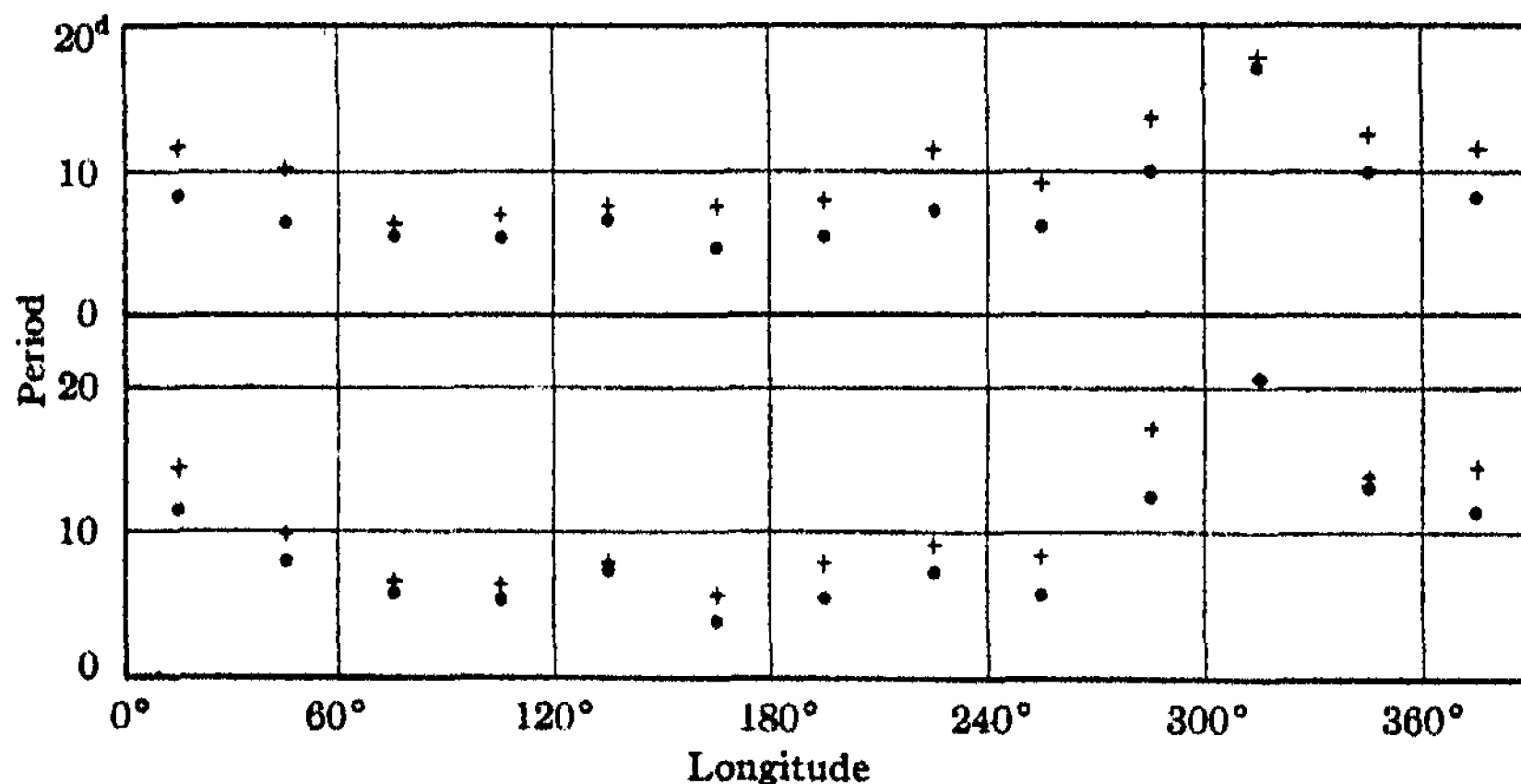


FIGURE 4

Dependence of period on galactic longitude; above, for the 336 known galactic Cepheids (periods  $> 1^d$ ; latitudes  $< \pm 30^\circ$ ), of all magnitudes (table 2); below, for the 227 galactic Cepheids with photographic magnitudes fainter than 10.0 (table 3). Crosses indicate mean periods and dots median periods in groups for  $30^\circ$  intervals in longitude.

value of the period,  $\bar{P}$ . In figure 4 the values of the median and mean periods are plotted.

Since sixty-eight per cent of the classical galactic Cepheids are fainter than the tenth magnitude, the material is given separately in table 3 for

these fainter and more distant variables; and the relation of period to longitude for them is plotted in the lower part of figure 4.

Whether we consider means or medians, consider all the variables or the fainter variables only, the conclusion is the same: in the interval of longitude that includes the galactic nucleus, say from  $270^\circ$  to  $360^\circ$ , the periods of the classical Cepheids average longer than in any other longitude. At first glance this result seems to be a clear confirmation of that already found from the study of the five fields near the galactic center, and of the result found for the Small Magellanic Cloud; but, in view of the numerous selection factors involved in the discovery and study of galactic Cepheids, the apparent confirmation should not be too strongly emphasized. It is probable that the scarcity of the longest-period Cepheids in the anticenter

TABLE 2  
LONGITUDE-DISTRIBUTION OF GALACTIC CEPHEIDS  
(All Magnitudes; Periods in Days)

$\lambda$	$15^\circ$	$45^\circ$	$75^\circ$	$105^\circ$	$135^\circ$	$165^\circ$	$195^\circ$	$225^\circ$	$255^\circ$	$285^\circ$	$315^\circ$	$345^\circ$
Number	22	27	22	18	16	21	26	17	76	34	21	36
$\bar{P}$	8.185	6.32	5.405	5.325	6.61	4.66	5.385	7.19	6.18	10.05	17.06	9.945
$\bar{P}$	11.49	10.15	6.28	6.76	7.57	7.59	7.97	11.51	9.15	13.70	17.89	12.59

TABLE 3  
LONGITUDE-DISTRIBUTION OF GALACTIC CEPHEIDS  
(Magnitudes Fainter than 10.0; Periods in Days)

$\lambda$	$15^\circ$	$45^\circ$	$75^\circ$	$105^\circ$	$135^\circ$	$165^\circ$	$195^\circ$	$225^\circ$	$255^\circ$	$285^\circ$	$315^\circ$	$345^\circ$
Number	13	19	14	14	12	15	21	6	50	21	16	26
$\bar{P}$	11.51	7.86	5.87	5.325	7.395	3.89	5.73	7.355	6.005	12.64	20.71	13.23
$\bar{P}$	14.51	9.89	6.67	6.53	8.01	5.83	7.91	9.30	8.51	17.30	20.68	14.02

region cannot be attributed solely to the nearness of the observer to the boundary of the Milky Way in that direction. The possibility however must be kept in mind that perhaps a part of the difference between center and anticenter is a matter of depth available for survey. But there should be little "boundary" selection in longitudes  $60^\circ$  and  $240^\circ$ , at right angles to the direction to the center; and we find in these longitudes a similar infrequency of the peculiarly long-period Cepheids such as predominate in MWF 184 and adjacent regions.

5. *Summary.*—The material presented in the tables and figures of the present note appear to confirm a phenomenon previously discovered in the Small Magellanic Cloud: in regions of highest star density the periods of classical Cepheids are distinctly longer on the average than at the borders of the system or in regions of intermediate star density.

The evidence now presented for the galactic Cepheids includes results

obtained from five rich variable star fields at or near the galactic center. In these regions classical Cepheids with periods of less than ten days are infrequent. Five of the eight of shortest period are bright or highly reddened Cepheids, not near the galactic nucleus but in the neighborhood of the Sun.

An examination of the distribution of the periods, magnitudes and longitudes of all the 340 known galactic Cepheids confirms the conclusions based on the study of the five nuclear fields.

The correlation of period length with concentration of stellar mass is being further investigated through additional studies of the classical Cepheids in the two Magellanic Clouds.

\* Cluster type Cepheids are peculiarly cosmopolitan in that they are the only variables known in the very low-density regions of the "galactic haze," far above and below the galactic plane; and they are also abundantly found not far from the centers of globular star clusters as well as in the galactic nucleus.

† The Cepheid X Sagittarii, the first variable in table 1, appears to be the "central" naked-eye star, bearing much the same relation to the galactic center as Polaris bears to the north pole; but  $\delta$  Ophiuchi, magnitude 4.37 and spectrum F5, is nearly as close to the center.

<sup>1</sup> These PROCEEDINGS, 26, 105-115 (1940); *Harvard Reprint* 192.

<sup>2</sup> For example, *Harvard Reprint* 105 (1934).

<sup>3</sup> Shapley and Swope, *Harv. Ann.*, 90, No. 5 (1934).

<sup>4</sup> See figure 3 of *Harvard Reprint* 58 (1929).

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## THE ABSORPTION OF BICARBONATE ION BY BARLEY PLANTS AS INDICATED BY STUDIES WITH RADIOACTIVE CARBON

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With the aid of radioactive carbon ( $C^{11}$ ) Ruben and Kamen<sup>1</sup> have found that many heterotrophic systems reduce<sup>2</sup> appreciable quantities of  $C^*O_2$  during respiration. Among the non-photosynthetic organisms investigated were yeast (baker's), *B. coli*, ground barley roots and ground liver (rat) tissue. The experiments involved the shaking of a distilled water suspension of the cells with a  $C^*O_2$ -air ( $\sim 5\%$   $C^*O_2$ ) mixture for periods varying from 5 to 100 minutes. The organisms were then freed of  $+4$  carbon (i.e.,  $CO_2$ ,  $HCO_3^-$ , etc.) by boiling vigorously with strong acid. In all cases the tissues were found to retain radioactive carbon. This indicated the presence of radiocarbon in oxidation states lower than  $+4$ .

The significance of this "assimilation" of  $C^*O_2$  is still uncertain. It is



possible that the apparent assimilation of  $C^{*}O_2$  is merely an interchange of  $CO_2$ . If this is the explanation, then the reactions producing  $CO_2$  in the respiratory process must be reversible. However, it is also possible that  $CO_2$  is being used in a synthesis of compounds important in cellular metabolism. Ruben and Kamen favor the latter proposal even though it seems contrary to existing ideas on the metabolism of heterotrophic cells. Experiments are in progress to decide whether the  $C^{*}O_2$  reduction is due to exchange or synthetic reactions.

The evidence pertaining to the assimilation of  $CO_2$  by barley roots in water suspension would seem to have a direct bearing on the study of electrolyte accumulation by root cells. It admits of the possibility of a considerable absorption of  $HCO_3^-$  ions by the non-photosynthetic root cells through a process which results in the reduction and storing up of the carbon in the form of new compounds. Although many investigations indicate that  $H^+$  and  $HCO_3^-$  ions may be intimately involved in the absorption process, the observations of the past have not been such as to suggest *the idea of absorption and assimilation of  $H_2CO_3$  or  $HCO_3^-$  by tissues which at the same time excrete relatively large quantities of carbonic acid.*

The mechanism whereby certain favored cations and anions are absorbed by actively metabolizing root cells and held in the vacuoles at concentrations far exceeding those in the bathing culture medium is still unelucidated. Many observations point to the fact that the seat of the accumulation reaction must lie in the cell protoplasm. Although ions must pass through the fibrillated cellulose layer comprising the cell wall and presumably through a plasma membrane before encountering the protoplasm, it does not seem possible that the net result of the accumulation process will be completely explained on the basis of simple diffusion and membrane equilibria.

An important approach to the study of the accumulation process would be the determination of the relative proportions in which oppositely charged ions are absorbed by the roots. Heretofore difficulty has existed in evaluating the rôles of  $H^+$  and  $HCO_3^-$  ions which are continuously excreted by the root as the result of the respiratory process. The possibility that  $HCO_3^-$  ion may play a part in the accumulation is suggested by the researches of Ruben and Kamen. These experiments, in addition to providing justification for further study regarding the nature of the "reduction" of  $CO_2$  by plant roots, also suggest a means (i.e., the use of radioactively labeled  $HCO_3^-$  ion) for deciding the question whether or not individual ions can be absorbed by the plant through a process of ion exchange between the root cells and the culture medium. The experimental background leading to this question is contained in the considerable store of information which has accrued as a result of studies of actively absorbing plant cells in artificially prepared nutrient solutions. Only the more pertinent part of this

information will be mentioned here. For a more complete survey of these data the reader is referred to a recent review by Hoagland.<sup>3</sup>

A salient characteristic of the absorbing root system is the *apparently* independent entry of cations and anions from the culture solution into the root cells. For example, with  $K_2SO_4$  culture solutions, the accumulation of  $K^+$  by barley roots is much greater than the accumulation of  $SO_4 =$ .<sup>3</sup> Likewise, in the case of  $Ca(NO_3)_2$  solutions, the accumulation of  $NO_3^-$  far exceeds the accumulation of  $Ca^{++}$ .<sup>3</sup> Moreover it has been observed that, for short intervals of time, the absorption of  $K^+$  from  $KHCO_3$  solutions is nearly equal to  $K^+$  absorption from  $KCl$  solutions, although in the one case no accumulation  $HCO_3^-$  in the roots can be demonstrated, and in the other case the absorption of  $Cl^-$  is often nearly equivalent to the absorption of  $K^+$ .<sup>4</sup>

Of the numerous mechanisms which have been proposed, probably the one which most satisfactorily accounts for the observed alterations in the bathing culture medium during active electrolyte absorption by plant roots is the exchange absorption theory of Brooks<sup>5</sup> (compare also Lundegårdh<sup>6</sup>). According to this theory the initial step in the absorption of either a cation or an anion involves an exchange of the ion of the culture solution for an ion of like charge which is held on the surface of or within the protoplasm. The exchange hypothesis is in harmony with the experimental observation that an excess accumulation of anions over cations is usually compensated for in the culture solution by  $HCO_3^-$  ions, and an excess accumulation of cations over anions is compensated for by  $H^+$  and other cations such as  $Na^+$ ,  $Ca^{++}$  and  $Mg^{++}$  from the plant.<sup>4</sup> It may be noted, however, that the investigations of Hoagland and Broyer<sup>4</sup> and others do not support the view that a simple  $H^+$  ion gradient mechanism between sap and external solution is involved.

Notwithstanding the fact that an exchange mechanism is in harmony with a number of observations, certain other observations are not easily explained. For example, in the case of barley roots, a certain *dependency* of cation absorption on anion absorption has been noted. Barley plants accumulate potassium much more readily from  $KCl$  solutions than from  $K_2SO_4$  solutions of comparable concentrations.<sup>4</sup> Moreover, in the case of tissue such as potato tuber, an approximately equivalent absorption of cations and anions is observed under a variety of conditions for salts such as  $KCl$  and  $KBr$ .<sup>7</sup>

An essential consequence of the exchange theory is the independency of cation and anion assimilation, at least within certain limits. Obviously this fact can never be demonstrated until the rôles of  $H^+$  and  $HCO_3^-$  ions, which are always present in actively absorbing root systems, have been evaluated. Until this has been accomplished experimentally, it can never be determined whether ions may enter the root cells independently through

exchange or must always enter as ion pairs of opposite sign. For instance, an apparently independent absorption of  $K^+$  from a  $K_2SO_4$  solution may actually be a simultaneous absorption of  $K^+$  and  $HCO_3^-$  ions in exactly equivalent amounts; likewise an apparently independent absorption of  $NO_3^-$  from a  $Ca(NO_3)_2$  solution in reality may be an equivalent uptake of  $H^+$  and  $NO_3^-$  ions. In the following experiments with radioactively labeled  $HCO_3^-$ , some of the hitherto encountered uncertainty regarding the behavior of that ion has been eliminated. The experiments were designed primarily to decide whether in the case of a  $KHCO_3$  culture solution, bicarbonate ion is reduced in amounts equivalent to the absorption of potassium ion.

*Experiment I.*—A preliminary absorption experiment was carried out on May 28. Young barley plants (approximately 3 weeks old) which had been grown by the technique of Hoagland and Broyer<sup>8</sup> were selected. The roots were so-called "low salt" roots, having an approximate K content of 40 milliequivalents per 100 g. of oven-dry material. The experiment was performed in the following manner. Twenty-one plants were decapitated about  $\frac{3}{4}$  inch above the root-stem plate and fixed by means of non-absorbent cotton in holes in the wooden cover of the culture solution vessel. The roots were thoroughly washed in distilled water and then immersed in the  $KHC^{*}O_3$  culture solution. The culture solution used was prepared as follows: 0.040 g. Merck's Reagent  $KHCO_3$  was dissolved in 15 ml. distilled water. This solution was shaken with 2 cc.  $C^{*}O_2$  (S.T.P.) in an evacuated bulb for 30 minutes at room temperature. The solution was then removed from the bulb and adjusted to 400 ml. with distilled water. The resulting culture medium was therefore very nearly 0.001 *N* in  $HCO_3^-$  ion. The volume of solution used in the experiment was 349 ml.

The system was aerated with a moderate stream of air throughout the period of absorption by the roots which was of exactly 64 minutes' duration. During the absorption all solution which exuded from the cut ends of the decapitated plants was collected by means of an eye-dropper and placed in a weighed weighing bottle containing 1 ml. of 0.1 *N* NaOH. At the end of the absorption period, the plants were removed from the culture medium and thoroughly washed with tap and distilled water. The plants were then cut into small pieces and placed in a distillation flask containing about 150 ml. distilled water. The  $CO_2$  ( $HCO_3^-$ ,  $CO_3^{--}$ , etc.) was liberated from the suspension by boiling with an excess of  $H_2SO_4$ . The distillate was collected in a small volume (15 ml.) of NaOH solution. Following the distillation the volume of the NaOH solution was determined accurately. This solution contained all of the carbon present in the plants as  $+4$  carbon. A measured aliquot of the solution was counted, and the total count of  $C^{*}$  present in the plant as  $HCO_3^-$  or  $H_2CO_3$  calculated. The roots were then removed from the distillation flask and boiled for about 10 minutes in 150

ml. of 95% ethyl alcohol. The alcohol extract was combined with the acid solution in the distillation flask. The resulting solution was filtered and adjusted to volume. A measured aliquot of this solution was counted and the total count for the solution calculated. This count corresponded to the  $C^*$  present in the plants as reduced carbon.<sup>9</sup> Finally the total count of the NaOH solution in the weighing bottle which contained the exudate was made. This count corresponded to the  $C^*$  present in the exudate both as  $+4$  carbon and as reduced carbon. The estimated volume of the exudate was 0.05 ml.

The distribution of radioactive carbon in the plant at the conclusion of the absorption period is given in the following table. The amounts of  $C^*$  are expressed in counts per minute.

Total $C^*$ (mostly $HCO_3^-$ ) originally available to plants from culture	= $1.18 \times 10^4$ counts/min.
Total $C^*$ found in plants as $HCO_3^-$ or $H_2CO_3$	= $1.60 \times 10^3$ "
Total $C^*$ found in plants as reduced carbon	= $4.56 \times 10^4$ "
Total $C^*$ found in exudate	= 12.7 "

Of the radioactive carbon originally available, the following percentages were retained by the plants:

Percentage retained as $HCO_3^-$ or $H_2CO_3$	= 0.14%
Percentage retained as reduced carbon	= 3.86%
Percentage in exudate	= 0.0011%
<hr/>	
Total percentage retained by plants	4.00%

*Experiment II.*—A much more complete absorption experiment was carried out on June 11. The plants used had been germinated May 14 and planted May 20. The K content of the roots of the 21 plants used in the experiment was 0.603 milliequivalent K. On the dry basis this corresponded to about 41 millequivalents per 100 g. The experiment was carried out in the same manner as I with two exceptions. The absorption period was 92 minutes and the volume of culture solution used (0.001 *N*  $KHCO_3$ ) was 361 ml. The following additional measurements were made:

- The volume of the exudate was measured (by weighing).
- The pH and total  $CO_2$  content of the culture solution at the beginning and at the end of the absorption period were determined.
- The loss of potassium from the culture solution during the absorption period was measured.

The results of measurements made on the culture solution before and after the 92-minute absorption period, together with the results of measurements on the plants and exudate, are presented in the following table:

Initial pH of culture solution	= 7.81 (23 °C.)
Final pH of culture solution	= 7.43 (23 °C.)
Initial CO <sub>2</sub> content of culture solution	= 0.362 millimol
Final CO <sub>2</sub> content of culture solution	= 0.209 millimol
Loss of CO <sub>2</sub> from culture solution (chiefly as HCO <sub>3</sub> <sup>-</sup> )	= 0.153 millimol
Total potassium absorbed by plants	= 0.142 milliequivalent
Initial activity of culture solution (total)	= 4.16 × 10 <sup>6</sup> counts/min.
Final activity of culture solution	= 1.15 × 10 <sup>6</sup> " "
Activity in plants represented by +4 carbon	= 0.091 × 10 <sup>6</sup> " "
Activity in plants represented by reduced carbon	= 2.050 × 10 <sup>6</sup> " "
Total activity in exudate	= 0.007 × 10 <sup>6</sup> " "
Total labeled carbon which entered plants	= 2.148 × 10 <sup>6</sup> " "
Volume of culture solution	= 361 ml.
Volume of exudate	= 0.353 ml.

From the data it is evident that, of the radiocarbon originally available in the culture solution, the following percentages were retained by the plants:

Percentage retained as HCO <sub>3</sub> <sup>-</sup> or H <sub>2</sub> CO <sub>3</sub>	= 0.22%
Percentage retained as reduced carbon	= 4.93%
Percentage in exudate	= 0.02%
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Total percentage retained by plants	= 5.1%

*Discussion of Results and Conclusions.*—In view of the differences in absorption period and volume of exudate, the results of Experiments I and II are in essential agreement. Radioactive carbon, present in the culture solution largely as HC\*O<sub>3</sub><sup>-</sup> ion, was definitely assimilated by the plant roots. Under the conditions of the experiments, 4–5 per cent of the originally available radiocarbon was retained by the tissue. Chemical examination of the tissue revealed that the absorbed C\* was chiefly present in the plants as reduced carbon. Only about 4 per cent of the radioactive carbon present was H<sub>2</sub>CO<sub>3</sub> or HCO<sub>3</sub><sup>-</sup>. A minute, although significant fraction of the absorbed C\* was exuded at the cut ends of the plants (0.3% in Experiment II).

The radioactivity in the exudate due to reduced carbon and that due to +4 carbon (HCO<sub>3</sub><sup>-</sup>) may be estimated as follows:

From previous experiments with barley roots it is known that concentrations of ions in the exudate rarely if ever exceed those in the sap expressed from the roots. Since the approximate volume of the sap in Experiment II was 14.9 ml., the concentration of labeled +4 carbon in the sap, expressed in counts per minute per ml., was  $6 \times 10^3$  counts/min./ml. From the data given above the total concentration of C\* in the exudate was equivalent to  $20 \times 10^3$  counts/min./ml. This indicates that a minimum of



70% of the radioactive carbon in the exudate was present in the reduced form.

Experiments comparable with Experiment II allow for a maximum of 0.2 millimol of  $\text{CO}_2$  being respired in the 92-minute absorption period. Owing to the exchange of  $\text{CO}_2$  between  $\text{HC}^*\text{O}_3^-$  of the culture medium and inactive  $\text{CO}_2$  excreted by the plants and  $\text{CO}_2$  of the air which was used for aeration, the isotopic mol fraction of  $\text{C}^*$  changed continuously throughout the absorption period. Thus in Experiment II the specific activity of the culture solution at the start of the absorption period was  $11.49 \times 10^6$  counts per minute per millimol  $\text{CO}_2$  and  $5.52 \times 10^6$  counts per minute per millimol at the end.

The calculation of the amount of bicarbonate ion assimilated by the roots depends on the estimation of the average specific activity of  $\text{HC}^*\text{O}_3^-$  at the outer surface of the protoplasm. On the assumption that the average specific activity at the protoplasmic surface was the same as the average specific activity calculated for the culture solution as a whole, the activity of the roots in Experiment II was equivalent to an assimilation of 0.025 millimol of  $\text{HCO}_3^-$ . However, the possibility of a lag in equilibrium between the region near the surface of the protoplasm and the rest of the culture solution is not inconceivable. Thus, this region may have been characterized by a lower specific activity than that calculated for the entire culture. This possibility would allow for the assimilation of a maximum of 0.06 millimol of  $\text{HCO}_3^-$  in Experiment II. When these values are compared with the value for the potassium absorption (0.142 millimol), it becomes evident that a several-fold absorption of  $\text{K}^+$  over  $\text{HCO}_3^-$  must have taken place.

This result speaks for the independent retention of cations by plant roots, a fact which lends substantial support to the exchange absorption theory. It should be emphasized, however, that this conclusion applies to the net result of the accumulation reaction which presumably takes place in the cell protoplasm. The results of these experiments in no way preclude the possibility that  $\text{K}^+$  and  $\text{HCO}_3^-$  may penetrate the root cell as far as the protoplasmic surface in chemically equivalent amounts.

Further, it should be recalled<sup>4</sup> that when  $\text{K}^+$  is accumulated from a  $\text{KHCO}_3$  solution, organic acids are synthesized and enter into equilibrium with the accumulated  $\text{K}^+$  ions.  $\text{K}^+$  and  $\text{HCO}_3^-$  ions might enter the protoplasm and after reaction with organic acid most of the  $\text{CO}_2$  be eliminated.

The total  $\text{CO}_2$  determinations on the culture solution of Experiment II show that 0.153 millimol was lost from the bathing culture medium. In view of the pH change and the fact that this quantity of  $\text{CO}_2$  could not have been reduced by the roots, one mechanism which would account for the loss would be an equivalent exchange of  $\text{K}^+$  ions of the culture solution



for  $H^+$  ions in the roots followed by the equivalent removal of  $H^+$  and  $HCO_3^-$  (as  $CO_2$ ) from the system through aeration.

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<sup>1</sup> Ruben, S., and Kamen, M. D., *Proc. Nat. Acad. Sci.*, **26**, 418-422 (1940).

<sup>2</sup> The terms oxidation and reduction usually imply a loss or gain of electrons. This idea has no theoretical significance when applied to the carbon compounds because the electron pairs are shared. While this limitation is generally recognized, these terms are widely used for the sake of convenience and brevity. We shall employ the usual terminology (cf. "The Oxidation States of the Elements and Their Potentials in Aqueous Solutions," W. M. Latimer, p. 118, Prentice-Hall, New York, 1938) which does not involve energy relationships or bond types, but refers to the kind of atom to which carbon is bonded.

<sup>3</sup> Hoagland, D. R., *Cold Spring Harbor Symposia Quant. Biol.* (in press), 1940.

<sup>4</sup> Hoagland, D. R., and Broyer, T. C., *Amer. Jour. Bot.*, **27**, 173-185 (1940).

<sup>5</sup> Brooks, S. C., *Trans. Faraday Soc.*, **33**, 1002-1006 (1937).

<sup>6</sup> Lundegårdh, H., *Die Naturwissenschaften*, **20**, 313-318 (1935).

<sup>7</sup> Steward, F. C., and Harrison, J. A., *Ann. Bot. N. S.*, **3**, 427-454 (1939).

<sup>8</sup> Hoagland, D. R., and Broyer, T. C., *Plant Physiol.*, **11**, 471-507 (1936).

<sup>9</sup> The efficiency of the isolation of +4 carbon and reduced carbon was verified by separate experiments. The boiling with alcohol resulted in the complete removal of active carbon from the solid part of the suspension.

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## A REMARK ON GENERAL FUCHSIAN GROUPS

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In the theory of the curves of genus 1 and the related elliptic functions a fundamental rôle is played by the modular group and its invariant functions (*modulfunctions*). Siegel generalized this group and these functions to the most general algebraic curves in two papers in *Annals of Mathematics*, **36**, 527-606 (1935), and in *Mathem. Annalen*, Bd. 116, pp. 617-657. In another paper in *Annals of Mathematics*, **41**, 488-494 (1940), Sugawara generalizes some of Siegel's results to a class of groups, which are the generalization of Poincaré's Fuchsian groups, and which the author calls "*general Fuchsian groups*." In this paper the author finds also a geometry, which, in his case, corresponds to Lobatschevski's geometry, so

useful in Poincaré's theory. We can arrive at Sugawara's geometrical theorems by means of elementary and general methods<sup>1</sup> and theorems.

Let  $A, B, C, D, X, Y$  be real matrices of order  $n$ ; we suppose that the determinant of  $Y$  is not equal to zero, and will write

$$Z = X + iY, \bar{Z} = X - iY.$$

We shall define a new matrix  $Z_1 = X_1 + iY_1$  (and  $\bar{Z}_1 = X_1 - iY_1$ ), by supposing

$$Z_1 = \begin{pmatrix} A & B \\ C & D \end{pmatrix} \cdot Z. \quad (1)$$

By (1) we mean that

$$Z_1 = (AZ + B)(CZ + D)^{-1} \text{ or } Z_1(CZ + D) = AZ + B. \quad (2)$$

If  $P, Q, R, S$  are matrices of order  $n$ , and if

$$Z_2 = \begin{pmatrix} P & Q \\ R & S \end{pmatrix} \cdot Z_1 = (PZ_1 + Q)(RZ_1 + S)^{-1}$$

we easily find that

$$Z_2 = \begin{pmatrix} H & K \\ L & M \end{pmatrix} \cdot Z = (HZ + K)(LZ + M)^{-1},$$

in which

$$\begin{pmatrix} H & K \\ L & M \end{pmatrix} = \begin{pmatrix} PA + QC & PB + QD \\ RA + SC & RB + SD \end{pmatrix}$$

is the product  $\begin{pmatrix} P & Q \\ R & S \end{pmatrix} \begin{pmatrix} A & B \\ C & D \end{pmatrix}$  of the preceding matrices (of order  $2n$ ).

Therefore:

*If a set of matrices  $\begin{pmatrix} A & B \\ C & D \end{pmatrix}$  of order  $2n$  forms a group, the corresponding transformations (1) or (2) also form a group.*

By differentiating (2) we get (by considering  $A, B, C, D$  as given and constant)

$$(A - Z_1C)dZ = (dZ_1)(CZ + D). \quad (3)$$

By considering the imaginary parts of the members of (2) we get

$$Y_1(C\bar{Z} + D) = (A - Z_1C)Y \text{ or } Y^{-1}(A - Z_1C)^{-1} = (C\bar{Z} + D)^{-1}Y_1^{-1} \quad (4)$$

By multiplying (3) and (4) we find that

$$Y^{-1}dZ = (C\bar{Z} + D)^{-1}Y_1^{-1}(dZ_1)(CZ + D) \quad (5)$$

and therefore also (since  $A, B, C, D, Y, Y_1$  are real)

$$Y^{-1}d\bar{Z} = (CZ + D)^{-1}Y_1^{-1}(d\bar{Z}_1)(C\bar{Z} + D). \quad (6)$$

By multiplying (5) and (6) we get finally:

$$Y^{-1}dZY^{-1}d\bar{Z} = (C\bar{Z} + D)^{-1}Y_1^{-1}(dZ_1)Y_1^{-1}(d\bar{Z}_1)(C\bar{Z} + D).$$

Therefore the two matrices

$$Y^{-1}(dZ)Y^{-1}(d\bar{Z}) \text{ and } Y_1^{-1}(dZ_1)Y_1^{-1}(d\bar{Z}_1) \quad (7)$$

are similar to each other and are also similar to

$$dZ(Y^{-1})(d\bar{Z})Y^{-1} = Y[Y^{-1}(dZ)Y^{-1}d\bar{Z}]Y^{-1} \text{ and } (dZ_1)Y_1^{-1}(d\bar{Z}_1)Y_1^{-1}. \quad (8)$$

Therefore

$$ds^2 = \text{Trace of } Y^{-1}(dZ)Y^{-1}(d\bar{Z}) = \text{Trace of } (dZ)Y^{-1}(d\bar{Z})Y^{-1} \quad (9)$$

is invariant under every transformation (1); and *every transformation (1) is a movement in the Riemannian geometry defined by the linear element (9).*

We have found this theorem only under the hypothesis that  $Y^{-1}$  exists (the determinant of  $Y$  is not equal to zero) and that (1) exists (the determinant of  $CZ + D$  is not equal to zero).

*This linear element (9) is real.* If  $Y^{-1}dZ = (u_{rs})$ , the element (9) is equal to  $\sum u_{rs}\bar{u}_{sr}$ . By interchanging  $r$  with  $s$ , we prove that it is equal to the conjugate element and that consequently it is real. If  $Z = (x_{rs} + iy_{rs})$  and  $Y^{-1} = y'_{rs}$  (in which  $y'_{rs}$  is the algebraic complement of  $y_{sr}$ ), an easy calculation proves that:

$$ds^2 = \sum y'_{rj}y'_{hi}(dx_{jh}dx_{ir} + dy_{jh}dy_{ir}) \quad (10)$$

I have demonstrated<sup>2</sup> that a group of movements in a Riemannian real *definite* geometry, which contains no infinitesimal transformations, is properly discontinuous.

*Consequently: If a group  $G$  of transformations (1) or (2) transforms into itself a region  $R$ , in which our element (10) is definite, and if it contains no infinitesimal transformations, it is properly discontinuous in  $R$ .*

For instance, let  $\Gamma$  be the group of the transformations (1) which satisfy Siegel's equations

$$A'C = C'A; B'D = D'B; A'D - C'B = E = \text{identity}$$

or the equivalent equations

$$AB' = BA'; CD' = DC'; AD' - BC' = E.$$

This group transforms into itself the region  $R$  of the *symmetrical matrices*  $Z = X + iY$ , for which  $Y > 0$  (or the quadratic form corresponding to  $Y$  is definite positive). And in this region it is transitive; for every point  $Z_1$

of  $R$ , we can find a transformation of  $\Gamma$  which carries  $Z_1$  into  $Z = iE$ . Since the linear element is invariant, and  $Y$  is the identity  $E$ , the linear element in the neighborhood of  $Z_1$  in  $R$  becomes equal to the trace of  $dZd\bar{Z}$  which is

$$\sum dz_{rs}d\bar{z}_{sr} = \sum (dx_{rs}^2 + dy_{rs}^2) \quad (\text{because } z_{sr} = \bar{z}_{rs})$$

and this form is obviously definite. We can also demonstrate, almost without calculations, that  $\Gamma$  is in  $R$  a group of movements for a real definite Riemannian geometry.  $\Gamma$  is transitive in  $R$ ; and the transformations of  $\Gamma$  which carry  $Z = iE$  into itself are obviously the transformations

$$\begin{pmatrix} A & B \\ -B & A \end{pmatrix} \quad (\text{if } A'B = B'A \text{ and } A'A + B'B = E).$$

This transformation carries the point  $iE + dZ$  into a point  $iE + dZ_1$ , and obviously

$$dZ = (A + iB)^{-1}(dZ_1)(A - iB).$$

Therefore the trace of  $dZ d\bar{Z}$  is invariant; since  $dZ$  is symmetric, this linear element is real and definite; and by means of a general theorem<sup>3</sup> we can deduce that  $\Gamma$  is in  $R$  a group of movements in a real definite Riemannian geometry.

The subgroups of  $\Gamma$  without infinitesimal transformations are called *general Fuchsian groups*, because they are the generalization of Poincaré's Fuchsian groups. We find consequently Sugawara's theorem:

*Every Fuchsian group is properly discontinuous in  $R$ .*

The most important Fuchsian group of order  $n$  is the modular group studied by Siegel, because it is the most important generalization of the classical modular group.

For  $n = 2$  I could demonstrate that  $\Gamma$  is the group of the real conformal transformations of the euclidean indefinite geometry defined by supposing  $ds^2 = dz_{11}dz_{22} - dz_{12}^2$ .<sup>4</sup> Therefore: *The group of the conformal real transformations in a euclidean indefinite geometry of three dimensions can be considered as a group of movements in a real definite Riemannian geometry of six dimensions.*

But for the modular group  $G$  Siegel went much farther. He determined its fundamental region by making use of Minkowski's results concerning the reduction of quadratic forms. It might be very interesting if one could succeed in finding Siegel's and Minkowski's theorems by making use of the general methods, by means of which one can very often find the fundamental region of a group of movements in a real definite Riemannian geometry.

*Some Remarks.*—I. If  $P = (p_{rs})$  is a symmetrical matrix, and  $Q$  another matrix, if

$$P_1 = Q'PQ,$$

let us consider the elements of  $P_1$  as functions of the elements  $p$  of  $P$  (while  $Q$  is constant); it is obvious that the absolute value of the Jacobian of these functions is equal to the absolute value of

$$|\det Q|^{-(n+1)} \quad (n = \text{order of } P, Q, P_1).$$

For the transformations of  $\Gamma$  the factor  $A - Z_1C$  which we find in (3) and (4) is equal to  $(ZC' + D')^{-1}$ , so that we can also write for the points of the corresponding region  $R$ :

$$dZ_1 = (ZC' + D')^{-1}(dZ)(CZ + D).$$

Let us consider the elements of  $Z_1$  as functions of the elements of  $Z$ . The absolute value of their Jacobian will be equal to

$$\Delta^{-(n+1)} \quad [\text{in which } \Delta = \text{determ}(CZ + D) = \text{determ}(ZC' + D')].$$

This Jacobian plays a fundamental rôle in Eisenhart's generalized series. In an analogous way we can find the discriminant of the preceding linear element.

II. Prof. Siegel has remarked that the geodesics of the definite geometry related to the group  $\Gamma$  are given by the following equation:

$$\begin{pmatrix} A & B \\ C & D \end{pmatrix} . Z = i \begin{pmatrix} p_1^t & 0 \dots 0 \\ 0 & p_2^t \dots 0 \\ \dots & \dots \\ 0 & 0 \dots p_n^t \end{pmatrix}$$

Here  $\begin{pmatrix} A & B \\ C & D \end{pmatrix}$  is any matrix of  $\Gamma$ ,  $t$  is a real variable,  $p_1, p_2, \dots, p_n$  are positive numbers  $\neq 1$ . This can be proved by an application of Cartan's idea for constructing the geodesics in a symmetrical space (Siegel).

III. By means of the transformation

$$W = (Z - iE)(Z + iE)^{-1} \quad (E = \text{identical matrix})$$

Sugawara transformed the preceding region, related to the group  $\Gamma$ , into a bounded region, and could therefore make use of Poincaré's methods in order to prove the convergence of some series.

IV. The group  $\Gamma$  may be *extended* by means of the *reflection*  $U$  defined by

$$Z_1 = -\bar{Z}.$$

This is obvious, because  $U^2 = E$  and, if  $\begin{pmatrix} A & B \\ C & D \end{pmatrix}$  is a transformation of  $\Gamma$ ,

$$U^{-1} \begin{pmatrix} A & B \\ C & D \end{pmatrix} U = \begin{pmatrix} A & -B \\ -C & D \end{pmatrix},$$

which is another transformation of  $\Gamma$ ; consequently the groups  $\Gamma$  and  $U^{-1}\Gamma U = U\Gamma U$  are identical.

<sup>1</sup> In the analytical part of his paper, Sugawara generalizes also Siegel's series. But it is not yet proved that Sugawara's functions are not identically equal to zero.

<sup>2</sup> Introduzione alla teoria dei gruppi discontinui e delle funzioni automorfe (Bologna, Zanichelli), page 126.

<sup>3</sup> Sugli spazii che ammettono un gruppo continuo di movimenti; *Annali di Matematica*, tomo 8, serie 3, 39-81 (1902).

<sup>4</sup> Introduzione alla teoria dei gruppi discontinui e delle funzioni automorfe (Bologna, Zanichelli), page 399.

## THE DISTANCE IN GENERAL FUCHSIAN GEOMETRIES

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In a preceding note printed in these PROCEEDINGS (p. 695, this issue) I occupied myself with the most general Fuchsian geometries; their geodetic lines were determined by Prof. Siegel. By using Siegel's theorem we can deduce the geodetical distance between two matrices  $Z = X + iY$ ,  $Z_1 = X_1 + iY_1$ , in which  $X, X_1, Y, Y_1$  are symmetrical matrices of order  $n$  and  $Y, Y_1$  are, moreover, definite positive. By  $E, N$  we indicate the identical and the null matrix. If  $P$  is a positive diagonal matrix of order  $n$ , its diagonal elements  $p_1, p_2, \dots, p_n$  are positive, and, according to Siegel's theorem, the point  $iP^t$  generates a geodetic line ( $t$  is a real parameter,  $P^t$  is the diagonal matrix, the elements of which are  $p_1^t, p_2^t, \dots, p_n^t$ ; we shall often write  $\sqrt{P}$  instead of  $P^{1/2}$ ). The linear element of the Fuchsian geometry is

$$ds^2 = \text{trace } \frac{1}{Y} dZ \frac{1}{Y} d\bar{Z} \quad (\bar{Z} = X - iY). \quad (1)$$

Therefore the distance between the points  $t = t_1, t = t_2$  of the preceding geodetic line is

$$\sqrt{(\log p_1)^2 + (\log p_2)^2 + \dots + (\log p_n)^2} |t_2 - t_1|. \quad (2)$$



If  $Z = X + iY$  is one of our matrices, the (non-modular) transformation  $\begin{pmatrix} 0 & -Y \\ E & -X \end{pmatrix}$  carries  $Z$  into  $iE$ ,<sup>1</sup> and  $\begin{pmatrix} -M & N \\ -N-M \end{pmatrix}$  is the most general linear transformation, which carries  $iE$  into itself ( $M, N$  matrices of order  $n$ ). Therefore

$$\begin{pmatrix} -M & N \\ -N-M \end{pmatrix} \begin{pmatrix} 0 & -Y \\ E & -X \end{pmatrix} = \begin{pmatrix} N & MY - NX \\ -M & NY + MX \end{pmatrix},$$

is the most general linear transformation which carries  $Z$  into  $iE$ . It is modular if and only if

$$N'M = M'N; \quad Y(M'M + N'N)X = X(M'M + N'N)Y; \quad E = (M'M + N'N)Y.$$

The second condition is a consequence of the third, and consequently can be disregarded. In order to simplify the third condition, let us choose an arbitrary non-singular matrix  $\Gamma$ , and put

$$N = C\Gamma^{-1}, \quad M = A\Gamma^{-1}.$$

The first condition becomes

$$C'A = A'C. \quad (3)$$

The third condition  $E = (\Gamma')^{-1}(A'A + C'C)\Gamma^{-1}Y$  is turned into the simpler condition

$$A'A + C'C = E \quad (4)$$

if we choose  $\Gamma$  in such a way that

$$Y = \Gamma\Gamma' \quad (5)$$

and this is obviously possible, because  $Y$  is positive definite. If  $X = 0$ ,  $Y = P$ , ( $P$  diagonal), we can suppose  $\Gamma = \Gamma' = \sqrt{P}$ . Therefore

$\begin{pmatrix} 1 & \sqrt{P} \\ \sqrt{P} & 0 \end{pmatrix}$  carries  $iP$  into  $iE$ , and consequently  $\begin{pmatrix} 0 & -\sqrt{P} \\ \frac{1}{\sqrt{P}} & 0 \end{pmatrix}$  carries

$iE$  into  $iP$ . Therefore

$$T = \begin{pmatrix} 0 & -\sqrt{P} \\ \frac{1}{\sqrt{P}} & 0 \end{pmatrix} \begin{pmatrix} N & MY - NX \\ -M & NY + MX \end{pmatrix} = \begin{pmatrix} \sqrt{P}A\Gamma^{-1} - \sqrt{P}(C\Gamma' + A\Gamma^{-1}X) \\ \frac{1}{\sqrt{P}}C\Gamma^{-1} & \frac{1}{\sqrt{P}}(A\Gamma' - C\Gamma^{-1}X) \end{pmatrix} \quad (6)$$

is the most general modular transformation, which carries  $Z$  into  $iP$  (under the conditions (3), (4), (5)).

If  $Z_1$  is another of our matrices, and  $P_1$  another positive diagonal matrix, and if  $A_1, C_1, \Gamma_1$  satisfy the equations analogous to (3), (4), (5), we shall deduce the most general modular transformation  $T_1$ , which carries  $Z_1$  into  $iP_1$  and which is given by a formula quite analogous to (6). Can  $T$  and  $T_1$  be identical? Or, in other words, can the same transformation carry  $Z$  into  $iP$  and  $Z_1$  into  $iP_1$ ? If we write that the coefficients of  $T_1$  are equal to the corresponding coefficients of  $T$ , and if we write also the equations analogous to (3), (4), (5), we get:

$$C_1' A_1 = A_1' C_1 \quad (3.1)$$

$$A_1' A_1 + C_1' C_1 = E \quad (4.1)$$

$$Y_1 = \Gamma_1 \Gamma_1' \quad (5.1)$$

$$A_1 = \sqrt{\frac{P}{P_1}} AK, \quad C_1 = \sqrt{\frac{P_1}{P}} CK \quad (7)$$

$$\sqrt{P} (C\Gamma' + A\Gamma^{-1} X) = \sqrt{P_1} (C_1\Gamma_1' + A_1\Gamma_1^{-1} X_1) \quad (8)$$

$$\frac{1}{\sqrt{P}} (A\Gamma' - C\Gamma^{-1} X) = \frac{1}{\sqrt{P_1}} (A_1\Gamma_1' - C_1\Gamma_1^{-1} X_1) \quad (9)$$

in which

$$K = \Gamma^{-1}\Gamma_1 \quad (\Gamma_1 = \Gamma K; \Gamma_1' = K'\Gamma'). \quad (10)$$

By virtue of (7) and (10), the (8), (9) are equivalent to:

$$C - QC\Delta = AH \quad (8.1)$$

$$-A + Q^{-1}A\Delta = CH, \quad (9.1)$$

in which

$$Q = P_1 P^{-1} = P^{-1} P_1; \Delta = KK'; H = \Gamma^{-1}(X_1 - X)(\Gamma')^{-1}. \quad (11)$$

( $H$  is symmetrical, since  $X_1, X$  are symmetrical.) By means of (7) we realize that (3.1) is equivalent to (3), and that (4.1) is equivalent to

$$A'Q^{-1}A + C'QC = \Delta^{-1}. \quad (4.2)$$

If  $P = E$ , and  $Q = P_1$  has the elements  $q_1, q_2, \dots, q_n$ , the distance from  $Z$  to  $Z_1$  (or from  $P$  to  $P_1$ ) is

$$\text{distance} = \sqrt{(\log q_1)^2 + (\log q_2)^2 + \dots + (\log q_n)^2}. \quad (12)$$

In order to calculate this distance, we must study the system of the five equations (3), (4), (4.2), (8.1), (9.1), in which  $A, C, Q$  are the three unknown matrices. We remark that  $\Delta = KK' = \Gamma^{-1}\Gamma_1\Gamma_1'\Gamma'^{-1} = \Gamma^{-1}Y_1(\Gamma')^{-1}$  is,

like  $Y_1$ , a positive symmetrical matrix. From (8.1), (9.1) we deduce that every element  $q_r$  of  $Q$  (which is also a diagonal positive matrix) satisfies the fundamental equation

$$\begin{vmatrix} E - q\Delta & H \\ -H & E - \frac{1}{q}\Delta \end{vmatrix} = 0. \quad (\text{I})$$

The demonstration is easy. By developing the equations (8.1) and (9.1) we get:

$$c_{rs} - q_r \sum_j c_{rj} \delta_{js} = \sum_j a_{rj} h_{js}; \quad -\sum_j c_{rj} h_{js} = a_{rs} - \frac{1}{q_r} \sum_j a_{rj} \delta_{js} \quad (8-9.2)$$

( $\delta_{js} = \delta_{sj}$ ,  $h_{sj} = h_{js}$ ,  $c_{rs}$ ,  $a_{rs}$  are the elements of  $\Delta$ ,  $H$ ,  $C$ ,  $A$ ).

For every value of  $r$  ( $r = 1, 2, \dots, n$ ) we obtain consequently a system of  $2n$  homogeneous linear equations, in  $2n$  unknowns  $c_{r1}, c_{r2}, \dots, c_{rn}, a_{r1}, a_{r2}, \dots, a_{rn}$ . The determinant of these equations (first member of I) must therefore be equal to zero.

By interchanging the rows or the columns, and by interchanging rows and columns, or by changing the sign of some rows, we easily realize that (I) is equivalent to:

$$\begin{vmatrix} E - \frac{1}{q}\Delta & H \\ -H & E - q\Delta \end{vmatrix} = 0, \text{ or } (\Gamma_1 2) \begin{vmatrix} E - q\Delta & H \\ H & -E + \frac{1}{q}\Delta \end{vmatrix} = 0 \quad (\text{I.1})$$

From (I.1) we deduce:

*The fundamental equation does not change if we write  $\frac{1}{q}$  in the place of the unknown  $q$  (reciprocal equation).*

From (I.2) we deduce another result. Let us consider  $2n$  new variables  $x_1, x_2, \dots, x_n$  and  $y_1, y_2, \dots, y_n$ . Let  $F(x)$  be the quadratic form of the  $x$ , the determinant of which is  $E - q\Delta$ , let  $F_2(y)$  be the quadratic form of the  $y$ , the determinant of which is  $-E + \frac{1}{q}\Delta$  and  $H(x, y)$  the bilinear form of the  $x, y$ , the determinant of which is  $H$ : a number  $q$  is a root of the fundamental equation if and only if the quadratic form

$$F = F_1(x) + F_2(y) + 2H(x, y)$$

(in  $2n$  variables  $x, y$ ) is singular. ( $H$  is symmetrical,  $\Delta$  is positive and symmetrical.) We shall prove: Under these hypotheses the roots of (I) are real and positive (and therefore (12) has a real meaning). We begin by remarking that (if  $Y$  and  $Y_1$  are given) the equations (5) and (5.1) do not determine completely  $\Gamma$  and  $\Gamma_1$ . It is known that we can change  $\Gamma, \Gamma_1$  in

such a way that  $K = \Gamma^{-1}\Gamma$  and  $\Delta = KK'$  become diagonal matrices (we know that  $\Delta$  is also positive). Obviously, by changing  $\Gamma$ ,  $\Gamma_1$  we must change  $H$  also, according to (11). If  $\delta_i$  are the (positive) elements of the (diagonal) matrix  $\Delta$ , we have obviously

$$F = \sum m_i x_i^2 - \sum n_i y_i^2 + 2 \sum h_{ij} x_i y_j$$

$$\left( m_i = 1 - q\delta_i; n_i = 1 - \frac{\delta_i}{q} \right)$$

1°. If  $F$  is singular, and  $q$  is real, it is not possible that all the coefficients  $m, n$  have the same sign. Let us suppose that the  $m, n$  are positive (if they were negative, we could study the form  $-F$ ). We get

$$F = (x_1')^2 + (x_2')^2 + \dots + (x_n')^2 - \varphi$$

in which

$$x_i' = \sqrt{m_i} x_i + \frac{1}{\sqrt{m_i}} \sum h_{ij} y_j$$

and

$$\varphi = \sum n_i y_i^2 + \sum_i \left( \frac{1}{\sqrt{m_i}} \sum_j h_{ij} y_j \right)^2.$$

The form  $\varphi$  does not depend upon the  $x'$ ; if  $F$  is singular,  $\varphi$  is consequently singular also, and therefore may be equal to zero even if the (real)  $y$  are not all equal to zero. But this is not possible, because  $n_i > 0$ .

If a root  $q$  were negative, all the  $m, n$  would be positive; which is not possible; therefore the roots are positive; and the differences  $\frac{1}{\delta} - q, q - \delta$  cannot have the same sign.

2°. The roots of (I) cannot be imaginary. If  $q = \rho (\cos \theta + i \sin \theta)$  is an imaginary root ( $\sin \theta \neq 0$ ), then

$$m_j = 1 - q\delta_j = 1 - r_j(\alpha + i\beta); n_i = 1 - \frac{\delta_i}{q} = 1 - s_j(\alpha - i\beta)$$

$$(r_j = \rho_j^\delta; s_j = \frac{\delta_j}{\rho}; \alpha = \cos \theta; \beta = \sin \theta \neq 0).$$

If  $F$  is singular, we can find  $2k < 4n$  linear real forms  $u_\alpha, v_\alpha$  in  $x, y$  such that

$$F = \sum (u_\alpha + iv_\alpha)^2 \quad (\alpha = 1, 2, \dots, k) (k < 2n).$$

By equating the imaginary parts we get

$$-\beta [\sum r_j x_j^2 + \sum s_j y_j^2] = 2 \sum u_\alpha v_\alpha \quad (j = 1, 2, \dots, n;$$

$$\alpha = 1, 2, \dots, k < 2n).$$

Since  $k < 2n$ , we can find real values of  $x, y$ , which are not all equal to zero, and which satisfy the  $k$  linear homogeneous equations  $v_1 = v_2 = \dots = v_k = 0$ . Therefore the second member, and consequently also the first member of the last identity will be equal to zero; and this is not possible, because  $\beta \neq 0, r > 0, s > 0$ . All the roots  $q_1, q_2, \dots, q_n, q_{n+1}, \dots, q_{2n}$  are consequently positive. We can suppose

$$q_1 \geq q_2 \geq \dots \geq q_n \geq q_{n+1} \geq \dots \geq q_{2n} \text{ or } q_1 \leq q_2 \leq \dots \leq q_n \leq \dots \leq q_{2n}.$$

From our theorems it follows that

$$q_{n+j} = \frac{1}{q_{n-j+1}} \quad (j = 1, 2, \dots, n).$$

The  $q_1, q_2, \dots, q_n$ , and therefore the distance (12), are determined and real; this distance is a symmetrical but non-algebraic function of the  $q_1, q_2, \dots, q_n$ . We must now study the system of the equations (3), (4), (4.2), (8.1), (9.1).

For the sake of simplicity we study only the most general case, and suppose that  $q_1, q_2, \dots, q_n$  are different from each other; these roots will be simple (except  $q_n$  if  $q_n = 1 = \frac{1}{q_{n+1}}$ ).

Since  $\Delta$  is diagonal, the equations (8.1), (9.1) or (8-9.2) become:

$$c_{rs}(1 - q_r \delta_s) = \sum_j a_{rj} h_{js} \quad (8.3)$$

$$a_{rs}(-1 + \frac{1}{q_r} \delta_s) = \sum_j c_{rj} h_{js} \quad (9.3)$$

If  $c_{rs} = \gamma_{rs}, a_{rs} = \alpha_{rs}$  is a solution ( $\gamma_{rs}, \alpha_{rs}$  not all equal to zero), also

$$c_{rs} = \rho_r \gamma_{rs}, a_{rs} = \rho_r \alpha_{rs} \quad (\rho_r \neq 0 \text{ arbitrary}) \quad (13)$$

is a solution. We deduce at once

$$\begin{aligned} \sum_j c_{rs} a_{js} - q_r \sum_j c_{rs} \delta_s a_{js} &= \zeta_{ri} \quad (\zeta_{ri} = \sum_{j, i} a_{rj} h_{js} a_{is}) \\ - \sum_j c_{rs} a_{js} + \frac{1}{q_i} \sum_j c_{rs} \delta_s a_{js} &= \sum_{ri} \quad (\sum_{ri} = \sum_{j, i} c_{rj} h_{js} c_{is}) \end{aligned} \quad (14)$$

in which  $\zeta_{ri}$  and  $\sum_{ri}$  are, like  $h_{rs}$ , symmetrical in  $r, i$ . Therefore

$$\left(\frac{1}{q_i} - q_r\right) c_{rs} a_{is} = \frac{1}{q_i} \zeta_{ri} + q_r \sum_{ri}$$

or

$$\sum_j c_{rs} a_{js} = Z_{ri} + \frac{q_r q_i}{1 - q_r q_i} (\zeta_{ri} + \sum_{ri}).$$

Therefore  $\sum_s c_{rs} a_{ts}$  is symmetrical in  $r, t$ . (Our demonstration is not valid if  $q_r q_t = 1$ ; but in this case  $r = t = n$ ,  $q_n = 1$  and our theorem is obvious.) Therefore

$$CA' = AC'. \quad (15)$$

From (14) we get also

$$\sum_s c_{rs} \delta_s a_{ts} = \frac{q_t}{1 - q_r q_t} (\delta_{rt} + \sum_{s \neq t} \delta_{st}) \quad (\text{if } q_r q_t \neq 1). \quad (16)$$

From (8.3), (9.3) we deduce

$$\begin{aligned} \sum_s c_{rs} c_{ts} - q_r \sum_s c_{rs} \delta_s c_{ts} &= \sum_{j,s} a_{rj} h_{js} c_{ts} \\ - \sum_s a_{rs} a_{ts} + \frac{1}{q_t} \sum_s a_{ts} \delta_s a_{rs} &= \sum_{j,s} c_{tj} h_{js} a_{rs} \end{aligned}$$

and, by subtracting,

$$\sum_s (c_{rs} c_{ts} + a_{rs} a_{ts}) = q_r \sum_s c_{rs} \delta_s c_{ts} + \frac{1}{q_t} \sum_s a_{ts} \delta_s a_{rs}. \quad (17)$$

By interchanging  $r$  with  $t$  and by subtracting, we get:

$$(q_r - q_t) \sum_s c_{rs} \delta_s c_{ts} + \left( \frac{1}{q_t} - \frac{1}{q_r} \right) \sum_s a_{ts} \delta_s a_{rs} = 0,$$

or, if  $r \neq t$  and consequently  $q_r \neq q_t$ :

$$q_r \sum_s c_{rs} \delta_s c_{ts} + \frac{1}{q_t} \sum_s a_{ts} \delta_s a_{rs} = 0 \quad (r \neq t). \quad (18)$$

From (17) and (18) we deduce that  $CC' + AA'$  is a diagonal matrix; from (13) we get that the  $r$ th element of the diagonal is

$$\rho_r^2 \sum_s (\gamma_{rs}^2 + \alpha_{rs}^2)$$

and we can choose the  $\rho$  in such a manner that all these elements are equal to 1, or that

$$E = CC' + AA'. \quad (19)$$

From (15), (19) it follows that  $\begin{pmatrix} A & -C \\ C & A \end{pmatrix}$  is a modular transformation [or that  $(C + iA)(C' - iA') = E$ ]. Therefore also the inverse transformation  $\begin{pmatrix} A' & C' \\ -C' & A' \end{pmatrix}$  is modular [or  $(C' - iA')(C + iA) = E$ ], and consequently

$$C'A = A'C; E = C'C + A'A;$$



the equations (3) and (4) are satisfied. From (3), (8.1), (9.1) we get:

$$C'C - C'QC\Delta = C'AH = A'CH = -A'A + A'Q^{-1}A\Delta$$

or

$$C'C + A'A = (C'QC + A'Q^{-1}A)\Delta$$

or

$$E = (C'QC + A'Q^{-1}A)\Delta$$

which is identical with (4, 2). All our equations are satisfied, and our problem is solved; in order to find the distance between  $Z$  and  $Z_1$  it is sufficient to make use of (12), if the  $q_i$  are the quoted roots of the fundamental equation (I). (It may be useful to consider  $\left(q + \frac{1}{q}\right)$  as the unknown of this equation.)

*Remarks.*—I. From (17) and from (8.1), (9.1) we deduce

$$CC' + AA' = QC\Delta C' + Q^{-1}A\Delta A'; \quad CC' + AA' = QC\Delta C' - Q^{-1}A\Delta A' + AHC' - CHA'.$$

Consequently  $AHC' = CHA'$ , and also  $AHC'$  is symmetrical; if  $C$  is not singular,  $C^{-1}AH$  is symmetrical too. (Don't forget that  $C^{-1}A$  is symmetrical.)

II. If we do not make use of the matrices  $\Gamma$ ,  $\Gamma_1$ , we can consider the transformations

$$\begin{pmatrix} \sqrt{P} A & -\sqrt{P} (CY + AX) \\ \frac{1}{\sqrt{P}} C & \frac{1}{\sqrt{P}} (AY - CX) \end{pmatrix}$$

which carry  $X + iY$  into  $iP$ , and are modular if

$$A'C = C'A; \quad A'A + C'C = Y^{-1}.$$

By using the preceding methods we find the equations

$$CY - QCY_1 = A(X_1 - X), \quad -AY + Q^{-1}AY_1 = C(X_1 - X);$$

and the fundamental equation (I) becomes:

$$\begin{vmatrix} Y - qY_1 & X_1 - X \\ X_1 - X & -Y + \frac{1}{q}Y_1 \end{vmatrix} = 0 \quad (\text{I, 3})$$

( $X_1$ ,  $X$  symmetrical;  $Y$ ,  $Y_1$  symmetrical and positive definite). This last equation can be written immediately, if one has given the matrices  $X + iY$ ,

$X_1 + iY_1$  and one wishes to calculate their distance by means of (12). It may perhaps be useful to consider  $q + \frac{1}{q}$  as the unknown of this equation.

It is easy to verify this result for Lobachevsky's geometry ( $n = 1$ ).

<sup>1</sup> By definition, if  $A, B, C, D$  are matrices of order  $n$ , the linear transformation  $\begin{pmatrix} A & B \\ C & D \end{pmatrix}$  carries  $Z$  into  $(AZ + B)(CZ + D)^{-1}$ .

<sup>2</sup> In order to study the particular cases when at least two of the  $q_i$  ( $i \leq n$ ) are equal to each other, it is necessary to study the group of the modular transformations which carry into itself every point of the geodetic line joining the points  $iE, iQ$ . The study of this group is interesting and not difficult.

## ABSTRACT DEFINITE BOUNDARY VALUE PROBLEMS

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In this note we describe the basis for an extensive further development of the abstract theory of boundary value problems initiated in a previous paper,<sup>1</sup> state some of the more important results of the extended theory and indicate some of its applications. A detailed discussion of the subject will appear elsewhere.

The present theory is aimed at those problems with which a semi-bounded symmetric form is associated—as, for example, the Dirichlet integral is associated with various problems involving Laplace's equation—and in consequence it is not as widely applicable as the theory developed in (A). However, there is great compensation for this in two important respects: first, where the present theory does apply, it provides at once a much more complete analysis than can be achieved on the basis of (A) alone; second, its basic concepts are much simpler than those of (A) and consequently the technical problems which arise in connection with its application to differential operators are considerably simpler.

We consider a bilinear non-negative definite symmetric form  $G(f, g)$  defined for every pair of elements  $f, g$  in a dense linear subset  $\mathfrak{G}$  of Hilbert space  $\mathfrak{H}$  and require that  $G$  be closed in the following sense: if  $\{f_n\}$  converges to  $f$  in  $\mathfrak{H}$  and  $\lim_{n \rightarrow \infty} G(f_n - f_m, f_n - f_m) = 0$ , then  $f$  is in  $\mathfrak{G}$  and

$$\lim_{n \rightarrow \infty} G(f_n - f, f_n - f) = 0.$$

In view of the fact that  $G$  is non-negative and closed,  $\mathfrak{G}$  itself is a Hilbert space with inner product  $G(f, g) + (f, g)$ , and when we speak of  $\mathfrak{G}$  as a space it is to be understood in this sense.

Before proceeding it is convenient to state an important result of Friedrichs<sup>2</sup> which plays a fundamental rôle in the theory under discussion.

**FRIEDRICHS' THEOREM.** *If  $G$  satisfies the requirements stated above, there exists one and only one self-adjoint transformation  $S = S(G)$  in  $\mathfrak{S}$  with  $\mathfrak{D}(S) = \subseteq \mathfrak{G}$  such that  $(f, Sg) = G(f, g)$  for all  $f$  in  $\mathfrak{G}$  and all  $g$  in  $\mathfrak{D}(S)$ . If  $H$  is an arbitrary transformation with domain in  $\mathfrak{G}$  such that  $(f, Hg) = G(f, g)$  for all  $f$  in  $\mathfrak{G}$  and all  $g$  in  $\mathfrak{D}(H)$ , then  $H \subseteq S$ .*

We now introduce our fundamental concept, an abstraction of the notion of boundary values, and state our basic theorem.

**DEFINITION 1.** *A closed linear transformation  $B$  from the Hilbert space  $\mathfrak{G}$  to a unitary or Hilbert space  $\mathfrak{N}$  is called a boundary operator on  $\mathfrak{G}$  if the following conditions are satisfied:*

- (1)  $\mathfrak{D}(B)$  is dense in the space  $\mathfrak{G}$ ;
- (2) the manifold of elements  $f$  of  $\mathfrak{D}(B)$  such that  $Bf = 0$  is dense in  $\mathfrak{G}$ ;
- (3)  $\mathfrak{N}(B)$  is dense in  $\mathfrak{N}$ .

*The space  $\mathfrak{N}$  is called the range space of  $B$ , and  $Bf$  is called the boundary value of  $f$ .*

It is worth while to note that if  $G$  is bounded above, then  $\mathfrak{G} = \mathfrak{S}$  and the only operator  $B$  satisfying the conditions of Definition 1 is identically zero. On the other hand if  $G$  is unbounded and  $\mathfrak{N}$  is an arbitrary unitary or Hilbert space, one can show that there exists a boundary operator on  $\mathfrak{G}$  with range-space  $\mathfrak{N}$ .

**THEOREM 1.** *Let  $B$  be a boundary operator on  $\mathfrak{G}$ . Then there exists one and only one pair of transformations  $T$  and  $N$  satisfying the following conditions:*

- (1)  $\mathfrak{D}(T) = \mathfrak{D}(N) \subseteq \mathfrak{D}(B)$ ;
- (2)  $\mathfrak{N}(T) \subseteq \mathfrak{S}$ ,  $\mathfrak{N}(N) \subseteq \mathfrak{N}$ ;
- (3) the transformation  $L$  in  $\mathfrak{S} \oplus \mathfrak{N}$  with domain the set of all vectors  $\{f, Bf\}$ , which takes  $\{f, Bf\}$  into  $\{Tf, Nf\}$  is self-adjoint;
- (4)  $(f, Tg) + (Bf, Ng) = G(f, g)$  for all  $f$  in  $\mathfrak{D}(B)$  and all  $g$  in  $\mathfrak{D}(T)$ .

To prove Theorem 1, one has only to note that, by virtue of the restrictions on  $B$ ,  $G$  can be regarded as a form defined on the set of all  $\{f, Bf\}$  in  $\mathfrak{S} \oplus \mathfrak{N}$  and in this sense satisfied all the conditions of Friedrichs' theorem. Application of the latter then yields at once the result stated.

The significance of Definition 1 and Theorem 1 and the direction of the analysis based on them is now best revealed by means of some concrete examples.

**EXAMPLE 1.** The space  $\mathfrak{S}$  is  $\mathfrak{L}_2(a, b)$ ,  $-\infty < a < b < \infty$ ;  $G(f, g) = \int_a^b (pf'g' + qfg)dx$ ,  $p > 0$ ,  $q \geq 0$ ;  $\mathfrak{N}$  is a two-dimensional unitary space and  $Bf = \{f(a), f(b)\}$ . In this case

$$Tf = -(pf')' + qf, \text{ and } Nf = \{-f'(a), f'(b)\}.$$

EXAMPLE 2. The space  $\mathfrak{S}$  is  $\mathfrak{L}_2(E)$ , where  $E$  is the portion of the  $(x, y)$ -plane bounded by a smooth closed curve  $C$ ;

$$G(f, g) = \int \int_E [p(f_x \bar{g}_x + f_y \bar{g}_y) + qf \bar{g}] dE, \quad p > 0, q \geq 0;$$

$\mathfrak{N}$  is the space  $\mathfrak{L}_2(C)$  and  $Bf$  is the value of  $f$  on  $C$ . Here  $Tf = -(pf_x)_x - (pf_y)_y + qf$ , and  $Nf = -p\partial f/\partial n$ .

EXAMPLE 3. The space  $\mathfrak{S}$  is the same as in Example 2;

$$G(f, g) = \int \int_E \nabla^2 f \nabla^2 \bar{g} dE,$$

where  $\nabla^2$  is Laplace's operator;  $\mathfrak{N}$  is the space  $\mathfrak{L}_2(C) \oplus \mathfrak{L}_2(C)$  and  $Bf = \{f(s), -\partial f/\partial n\}$ . Here  $T$  is the biharmonic operator,  $Tf = \partial^4 f/\partial x^4 + 2\partial^4 f/\partial x^2 \partial y^2 + \partial^4 f/\partial y^4$ , and  $Nf = \{-\partial \nabla^2 f/\partial n, -\nabla^2 f(s)\}$ .

In describing these examples we have for the sake of brevity passed over various function-theoretic details such as the definition of the set  $\mathfrak{G}$ , the precise conditions on the curve  $C$  in Examples 2 and 3, and the definition of boundary values and normal derivatives. These matters, however, will all be dealt with in subsequent papers on the applications of the present theory.

THEOREM 2. *The transformation  $T$  of Theorem 1 has domain dense in  $\mathfrak{S}$  and thus  $T^*$  exists. The relation  $T \supseteq T^*$  holds and  $\mathfrak{D}(T^*)$  consists of those and only those elements  $f$  of  $\mathfrak{D}(T)$  such that  $Bf = Nf = 0$ . The operator  $T^*$  is Hermitian and non-negative definite.*

Two cases now arise according as the domain of  $T^*$  is or is not dense in  $\mathfrak{S}$ . In the former case  $T^*$  is symmetric,  $\tilde{T}$  exists and is identical with  $T^{**}$ ; in the latter,  $\tilde{T}$  does not exist.

THEOREM 3. *Let  $\mathfrak{A}$  be the manifold of zeros of  $B$ ,  $\mathfrak{M}$  the closure in  $\mathfrak{S}$  of  $\mathfrak{G} \ominus \mathfrak{A}$ , and  $S = S(G)$  the operator in  $\mathfrak{S}$  associated with  $G$  by Friedrichs' theorem. Then  $\mathfrak{D}(T^*)$  is dense in  $\mathfrak{S}$  if and only if  $\mathfrak{M} \cdot \mathfrak{D}(S) = \mathfrak{D}$ .*

In the remainder of this note we assume that the condition of Theorem 3 is satisfied; this is the case in the examples given above and in all similar ones of which we have been able to conceive.

We now bring Theorem 1 into correlation with the theory of  $(A)$ .

THEOREM 4. *Let  $A$  be the transformation with domain  $B(T)$  which takes  $\{f, Tf\}$  into  $\{Bf, Nf\}$ . Then  $A$  is a reduction operator for  $T$  in the sense of  $(A)$ . The range-space of  $A$  is  $\mathfrak{N} \oplus \mathfrak{N}$ , and the unitary operator  $W$  in  $\mathfrak{N} \oplus \mathfrak{N}$  associated with  $A$  takes  $\{h, k\}$  into  $\{k, -h\}$ .*

Thus, when the condition of Theorem 3 is satisfied, the results of  $(A)$  apply to yield an analysis of the situation described in Theorems 1 and 2 with reference to the problem of characterizing by boundary conditions those self-adjoint transformations  $S$  such that  $T^* \subseteq S \subseteq T$ . We shall not, however, discuss this aspect of the theory in the present note; its nature is

adequately indicated in an earlier communication<sup>3</sup> dealing with differential operators of the type appearing in Example 2 above.

Rather we wish to emphasize here that portion of the theory which stems directly from Theorem 1 and which provides the machinery for the operational treatment of many important problems in differential equations which had no precise interpretation in terms of the concepts of (A) alone. Beside thorough examination of the various cases which arise under Definition 1 and Theorem 1, which is, of course, technically necessary, this theory comprises (1) a careful analysis of the abstract analogues of the Dirichlet and Neumann problems of potential theory, of the so-called mixed boundary value problem, and of what may be termed abstract boundary value problems of the third kind; (2) a detailed study of the homogeneous boundary conditions related to each of the above problems, and of the characteristic value problems involving them; (3) the development of an elementary theory of variations for forms  $G$  satisfying the conditions stated at the beginning of this note and certain additional requirements, with reference to those variational problems which are equivalent to important boundary and characteristic value problems.

Applied to ordinary differential forms and operators this theory yields at once a considerable portion of the classical theory of boundary and characteristic value problems; indeed, it is not yet determined how far one can proceed abstractly in this direction. Moreover, applied to partial differential forms and operators, the theory achieves a considerable generalization and refinement of known results in several directions; this assertion is true in particular with reference to Examples 2 and 3 above, and will be amplified in a subsequent paper on differential operators. Finally, the abstract theory clarifies to a great extent many familiar analogies between boundary value problems involving various kinds of differential operators and systems of differential operators; it now becomes possible to regard these similar problems no longer merely as analogous one to the other but rather as special instances of the same abstract problem.

To conclude, and to amplify and illustrate the preceding general remarks, we state precisely some of our results, leaving to the reader their interpretation in terms of the examples given above.

First, let us consider the boundary condition  $Bf = 0$ , which is of special importance in all of the examples we have given and in many others. To begin we recall a result of Friedrichs.<sup>2</sup> If  $H$  is a closed linear non-negative definite symmetric transformation in  $\mathfrak{H}$ , the form  $(f, Hg)$  has a unique closed linear extension  $G_0$  in  $\mathfrak{H}$  which is determined by completing the space  $\mathfrak{D}(H)$  with inner product  $(f, Hg) + (f, g)$ . If  $S(G_0)$  is the self-adjoint transformation associated with  $G_0$  by Friedrichs' theorem above, then  $S(G_0) \supseteq H$ . We call  $S(G_0)$  Friedrichs' extension of  $H$ .

**THEOREM 5.** *Let  $S$  be the contraction of  $T$  with domain the set of elements*



$f$  of  $\mathfrak{D}(T) \cdot \mathfrak{D}(B)$  such that  $Bf = 0$ . Then  $S$  is Friedrichs' extension of  $T^*$ .

THEOREM 6. Let  $\mathfrak{A}$  be the orthogonal complement in the space  $\mathfrak{G}$  of the manifold of zeros of  $B$ . Then a necessary and sufficient condition that the relations  $S \subseteq T$  hold is that for some constant  $k$  and all  $f$  in  $\mathfrak{A}$ , we have

$$(f, f) \leq k^2(Bf, Bf).$$

Now let us turn to the problem of solving the system

$$\tilde{T}f = 0, Bf = h, \quad (*)$$

where  $h$  is some element of  $\mathfrak{R}$ , noting that when  $G(f, f)$  is the Dirichlet integral and  $Bf$  the ordinary boundary value of  $f$  (cf. Example 2 above), this is just the Dirichlet problem. In the projected, comprehensive discussion of this subject, this important problem is treated in detail; here we merely state one result.

THEOREM 7. Let the manifold  $\mathfrak{M}$  of singularities of  $G$  have a finite dimension number and belong to the domain of  $B$ . Let  $G$  be positive definite in  $\mathfrak{G} \ominus \mathfrak{M}$ . Then the system  $(*)$  has a solution  $f$  for every  $h$  in  $\mathfrak{R}(B)$ . This solution is unique if and only if the transformation  $S$  of Theorem 5 has an inverse.

THEOREM 8. (Dirichlet principle.) Let the hypothesis of Theorem 7 be satisfied. Let  $h$  be an arbitrary element of  $\mathfrak{R}(B)$  and let  $\mathfrak{F}(h)$  be the set of all  $f$  in  $\mathfrak{D}(B)$  such that  $Bf = h$ . Then  $G(f, f)$  has a minimum on  $\mathfrak{F}(h)$  which it attains if and only if  $f$  is a solution of the system  $(*)$ .

Finally, let us consider the condition  $Nf = 0$  and the abstract Neumann problem. Here again we restrict our attention to special results.

THEOREM 9. Let  $B$  be bounded and let  $S$  be the contraction of  $T$  whose domain consists of those and only those elements  $f$  of  $\mathfrak{D}(T)$  such that  $Nf = 0$ . Then  $S$  is the transformation  $S(G)$  associated with  $G$  by Friedrichs' theorem.

THEOREM 10. Let  $B$  be bounded and let the hypothesis of Theorem 7 be satisfied. Let  $\mathfrak{B}$  be the manifold in  $\mathfrak{R}$  of boundary values of elements of  $\mathfrak{D}(T)$  such that  $Tf = 0, Nf = 0$ . Then the system

$$Tf = 0, Nf = h$$

has a solution for every  $h$  in  $\mathfrak{R} \ominus \mathfrak{B}$ . This solution is unique if and only if the transformation  $S$  of Theorem 9 has an inverse and in this case  $\mathfrak{R} \ominus \mathfrak{B} = \mathfrak{R}$ .

<sup>1</sup> J. W. Calkin, *Trans. Amer. Math. Soc.*, **45**, 369-442 (1939). This paper will hereafter be referred to as (A).

<sup>2</sup> K. Friedrichs, *Math. Ann.*, **109**, 465-487 (1934).

<sup>3</sup> J. W. Calkin, these PROCEEDINGS, **25**, 201-206 (1939).



# UNSTABLE MINIMAL SURFACES OF HIGHER TOPOLOGICAL TYPES

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1. We are ultimately concerned with extending the calculus of variations in the large to multiple integrals. The problem of the existence of minimal surfaces of unstable type contains many of the typical difficulties, especially those of a topological nature. In the case of  $m$  contours ( $m > 1$ ) new difficulties appear not found either in the general theory when  $m = 1$  or in the extensive minimum theory when  $m > 1$ . The case  $m = 2$ , however, appeared to contain the essentially new difficulties, and for simplicity we present this case, leaving the extensions for later papers.

2. *The Curves  $g_0$  and  $g_1$ .*—We shall be concerned with two simple closed rectifiable curves  $g_0$  and  $g_1$  in  $n$ -space separated by an  $(n - 1)$ -plane and satisfying the chord arc condition. Under the chord arc condition the ratio of an arbitrary chord length to the lesser of the two corresponding arc lengths shall be bounded from 0. In certain aspects of our work not all these hypotheses are needed. For example, in the homotopy theorem the curves  $g_0$  and  $g_1$  need only be simple and closed.

3. *Canonical Pairs  $(\varphi, h)$ .*—The curves  $g_k$ ,  $k = 0, 1$ , shall each be of the form  $x = g(t)$ , in vector notation, where  $0 \leq t \leq 2\pi$  with  $t$  proportional to the arc length and  $g(t)$  with a period  $2\pi$  in  $t$ . We shall use other representations of  $g$  of the form  $g(h(\alpha))$  where  $h(\alpha)$  is a non-decreasing transformation of  $\alpha$  with the property that  $h(\alpha + 2\pi) \equiv h(\alpha) + 2\pi$ . The case where the discontinuities of  $h(\alpha)$  occur exclusively at a set of points  $\alpha$  which are congruent mod  $2\pi$  to a constant  $c$  is called *degenerate*. Let  $h(\alpha)$  and  $k(\alpha)$  be two transformations and  $r$  any rational integer. The minimum of the Lebesgue integral

$$\frac{1}{2\pi} \int_0^{2\pi} (h(\alpha) - k(\alpha) + 2\pi r)^2 d\alpha$$

with respect to  $r$  will be denoted by  $[h, k]$ . We regard  $[h, k]$  as a distance in a metric space  $I$ , proving that  $[h, k]$  satisfies the triangle axiom and identifying transformations  $h$  and  $k$  if  $[h, k] = 0$ . The space  $I$  is compact.

We choose three constants  $\alpha_1 < \alpha_2 < \alpha_3$  on the interval  $0 \leq \alpha < 2\pi$ . Continuous transformations  $\varphi(\alpha)$  which satisfy the condition  $\varphi(\alpha_i) = \alpha_i$  are termed *restricted* and form a subspace  $I^*$  of  $I$ . Such transformations will be denoted by Greek letters. A directly conformal 1 to 1 transformation of the disc  $u^2 + v^2 \leq 1$  into itself induces a transformation  $T(\theta)$  of the point  $(1, \theta)$  on the circle  $r = 1$  into a point  $(1, T(\theta))$ . We term  $T(\theta)$  a *Mobius*

*transformation.* A restricted transformation  $\varphi(\alpha)$  and a general transformation  $h(\alpha)$  such that  $\varphi(\alpha) \equiv h(T(\alpha))$  are said to be *connected* by  $T$ . A Mobius transformation  $T$  connecting a restricted transformation  $\varphi$  with a continuous transformation  $h$  is uniquely determined on  $I$  by  $\varphi$  and  $h$ , and maps a subspace of  $I^* \times I$  continuously onto a subspace of  $I$ . The space of Mobius transformations completed with the degenerate transformations is a compact subspace of  $I$ . Pairs  $(\varphi, h)$  are termed *canonical* if  $\varphi$  is restricted, and if  $h$  is either connected with  $\varphi$  or is degenerate. The limit  $(\varphi, h)$  on  $I^2$  of a sequence of canonical pairs is canonical whenever  $\varphi$  is continuous.

4. *The Dirichlet Sum D.*—Corresponding to a vector  $p(\theta)$  defining a simple closed curve, we introduce the Douglas functional

$$A(p) = \frac{1}{16\pi} \int_G \int \frac{[p(\alpha) - p(\beta)]^2}{\sin^2 \frac{(\alpha - \beta)}{2}} d\alpha d\beta \quad (1)$$

with  $G$  the parallelogram  $(0 \leq \alpha \leq 2\pi), (-\pi \leq \alpha - \beta \leq \pi)$ .

Let  $B$  be a ring region bounded by a circle  $C_0: r = 1$  and a circle  $C_1: r = \rho, 0 < \rho < 1$ . Let  $H(u, v)$  be the harmonic surface in vector form defined over  $B$  with the respective boundary values

$$p_k(\theta) = g_k[h_k(\theta)] \quad (k = 0, 1) \quad (2)$$

where  $h_k(\theta)$  is a continuous transformation. The usual Dirichlet integral sum for  $H(u, v)$  will be denoted by  $D(p_0, p_1, \rho)$ . Its value [6] is

$$D(p_0, p_1, \rho) = A(p_0) + A(p_1) + R(p_0, p_1, \rho) \quad (0 < \rho < 1) \quad (3)$$

where  $R$  is given in [2], p. 280. We define  $D(p_0, p_1, \rho)$  when  $\rho = 0$  or when  $h_0$  or  $h_1$  is degenerate, by (3), noting that  $R$  is defined in these cases in [2].

The function  $D$  and space of sets  $(p_0, p_1, \rho)$  is inadequate because the degenerate points offer trivial minima, the space is not simply connected,  $D$  is not weakly upper reducible (defined in [1]) at degenerate points, and because the representation of minimal surfaces by critical sets is needlessly multiple, thereby complicating the topology.

5. *The Space  $\Pi$  and function  $W(P)$ .*—A product of metric spaces will here be metricized by a distance function which is the sum of the component distance functions. Let  $J$  denote the interval  $0 \leq \rho < 1$ . The space  $\Pi$  shall consist of sets or points  $P = (\varphi_0, \varphi_1, h_0, h_1, \rho)$  in which  $\rho$  varies on  $J, h(0) = 0$ , and  $(\varphi_k, h_k), k = 0, 1$ , is a canonical pair of transformations. The pair  $\{\varphi_0, \varphi_1\}$  will be termed the *restricted projection* of  $P$  and will be regarded as a point on  $I^2$ . When  $\rho = 0$ , two points  $P$  with the same restricted projection are identified. When  $\rho > 0$  points  $P$  are identified as on  $I^4 J$ . Let  $d(P, Q)$  be the distance between  $P$  and  $Q$  as points of  $I^4 J$ . With  $Q = (\psi_0, \psi_1, k_0, k_1, \sigma)$  let

$$\delta(P, Q) = [\varphi_0 \psi_0] + [\varphi_1 \psi_1] + \rho + \sigma.$$

The points  $P$  form a space  $\Pi$  on which our final choice of distance function  $PQ$  shall be the minimum of  $d(P, Q)$  and  $\delta(P, Q)$ . The usual axioms are satisfied by  $PQ$ .

Set

$$q_k(\theta) = g_k[\varphi_k(\theta)] \quad A(g_k, \varphi_k) = A(q_k).$$

To define  $W(P)$  we use (2) and set

$$W(P) = A(g_0, \varphi_0) + A(g_1, \varphi_1) + R(p_0, p_1, \rho).$$

As one sees  $W(P) > D$  when  $h_0$  or  $h_1$  is degenerate. We show that  $W(P)$  is regular at infinity, and boundedly compact in the sense of [1]. Moreover, the connectivity  $R_0$  of the subspace on which  $W$  is finite is 1, and the other connectivities are 0. A point  $P$  at which  $\rho > 0$  is termed *differentially critical* when the boundary vectors (2) define a ring minimal surface over  $B$ ; or, if  $\rho = 0$ , when these vectors define disc minimal surfaces. The homotopy theorem states that a homotopic critical point is differentially critical.

We show that  $W(P)$  is weakly upper reducible at each ordinary or degenerate point, and if  $g'_k(t)$  satisfies a Lipschitz condition at critical points. *The general theory thus applies with the consequent relations between the critical sets classified as to type members.* The following special theorem is provable by a limiting process [11] under the original weak hypothesis. These hypotheses are weaker than those of Shiffman [4] in the case of one contour.

**THEOREM.** *If  $g_0$  and  $g_1$  bound a ring minimal surface belonging to a minimizing set of critical points of  $W$ , there either exists a ring minimal surface of non-minimizing type bounded by  $g_0$  and  $g_1$ , or else a disc minimal surface of non-minimizing type bounded by  $g_0$  or by  $g_1$ .*

If  $g_0$  and  $g_1$  possess convex plane projections, the first alternative of the theorem alone holds.

When  $\rho = 0$ ,  $W$  is the sum of the functions  $A(g_k, \varphi_k)$  defined on the product space  $I^* \times I^*$ . Critical points in this subspace correspond to two disc minimal surfaces. Let a function  $f(x) + f(y) = F$  be defined on a product of two metric spaces of points  $x$  and  $y$ , respectively. Are the type numbers of critical points of  $F$  obtainable by summation from those of  $f(x)$  and  $f(y)$  in the natural way, that is, as in the case where  $f(x)$  and  $f(y)$  are non-degenerate forms? This question has been answered affirmatively by A. E. Pitcher under conditions which admit a wide range of applications. Pitcher's result will presently be published.

<sup>1</sup> Morse and Tompkins, "The Existence of Minimal Surfaces of General Critical Types," *Ann. Math.*, 40 (1939). Corrections for this paper will appear in *Ann. Math.*, Jan., 1941.

<sup>2</sup> Morse, "The First Variation in Minimal Surface Theory," *Duke Math. Jour.*, 6 (1940).

<sup>3</sup> Morse and Tompkins, "Minimal Surfaces of Unstable Type," *Bull. Amer. Math. Soc.*, **46**, 222 (1940). This is an abstract of the present paper presented at the Columbus meeting in Dec., 1939. See also *Science*, **91**, 457 (1940) for a fuller abstract.

<sup>4</sup> Shiffman, "The Plateau Problem for Non-relative Minima," *Ann. Math.*, **40** (1939).

<sup>5</sup> Courant, "The Existence of Minimal Surfaces of Given Topological Structure under Prescribed Boundary Conditions," *Acta Mathematica*, **72** (1940).

<sup>6</sup> Douglas, "The Problem of Plateau for Two Contours," *Jour. Math. and Physics*, **10** (1931).

<sup>7</sup> Douglas, "Minimal Surfaces of Higher Topological Structure," *Ann. Math.*, **40**, 205-298 (1939).

<sup>8</sup> Radò, "On the Problem of Plateau," *Ergebnisse der Mathematik*, **2**, 75 (1933).

<sup>9</sup> Morse, *Functional Topology and Abstract Variational Theory*, Gauthier-Villars, Paris.

<sup>10</sup> Morse, "Rank and Span in Functional Topology," *Ann. Math.*, **41**, 419-454 (1940).

<sup>11</sup> Morse and Tompkins, "Minimal Surface of Unstable Type by a New Mode of Approximation." To be published in *Ann. Math.*, **42** (1941).

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